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AMINO ACID	2	3	4	5	6	7	8	9	10	11	12
A	1.0	235.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
C	2.2	6818.2	1.9	0.3	0.4	1.0	0.8	0.7	0.8	0.9	0.7
D	3.9	6818.2	7.0	2.4	0.9	1.4	7.8	4.3	2.2	26.0	1.6
E	1.4	6818.2	12.0	3.8	0.5	1.0	7.0	5.0	1.4	31.0	2.2
F	0.7	1.7	0.4	0.6	0.3	0.8	12.4	1.9	0.6	36.0	0.4
G	0.7	1570.5	4.2	0.8	2.0	1.0	1.1	2.0	1.5	1.4	1.1
H	2.4	6818.2	0.9	2.1	0.7	0.9	2.9	0.5	2.2	4.0	1.1
I	2.0	6.4	0.4	0.9	0.8	0.7	0.8	1.2	0.7	11.7	1.6
K	1.2	6818.2	0.4	2.7	30.0	1.3	13.3	20.0	1.1	23.0	1.1
L	1.5	15.4	0.4	0.5	0.6	1.8	1.4	1.0	0.9	4.2	0.8
M	1.2	8.3	1.4	0.5	0.3	0.7	3.3	0.8	0.8	4.5	0.7
N	2.0	4159.1	0.7	2.6	0.2	1.2	0.6	1.2	0.9	5.0	1.4
P	7.6	83.6	544.0	1.7	70.8	3.0	1.5	0.8	0.7	5.4	1.2
Q	0.7	6818.2	0.9	4.2	0.6	1.6	1.9	8.5	0.6	5.4	0.6
R	1.5	6818.2	0.4	2.7	18.3	1.1	17.2	14.0	1.3	18.0	1.4
S	1.5	6818.2	0.7	0.4	0.8	1.0	0.7	1.6	2.6	1.7	1.2
T	0.7	371.6	0.8	1.3	1.2	0.7	0.6	1.7	3.0	6.9	0.8
V	1.1	14.1	0.5	1.0	1.0	0.8	0.9	1.2	0.6	6.2	1.8
W	3.2	1.2	0.6	1.8	1.5	0.7	2.8	1.5	1.2	7.8	0.6
Y	1:2	1.0	0.6	0.7	0.6	0.5	3.2	0.4	0.4	10.2	0.4

(57) Abstract

The present invention provides peptides of human myelin oligodendrocyte glycopeptide (MOG), an autoantigen related to demyelinating autoimmune diseases. The present invention also provides compositions including MOG peptides or mixtures of MOG peptides and myelin basic peptide (MBP) peptides. Also provided are therapeutic compositions useful for diagnosing and treating autoimmune diseases, and methods of treating multiple sclerosis using such therapeutic compositions.

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MYELIN OLIGODENDROCYTE GLYCOPROTEIN PEPTIDES AND USES THEREOF

BACKGROUND OF THE INVENTION

The invention is directed to autoantigens and their relevant epitopes. More specifically, the invention concerns myelin oligodendrocyte glycoprotein (MOG) and the peptide regions thereof useful in diagnosis, treatment, and prevention of autoimmune conditions. Further, methods of screening for, and developing therapeutics useful in the treatment of, autoimmune disease are also disclosed.

Autoimmune diseases are a significant human health problem and are relatively poorly understood. As there is no microbial or viral culprit apparently directly responsible, prevention, treatment and diagnosis of such diseases must be based on the etiology of the disease. This invariably involves a complex series of reactions of endogenous metabolic intermediates, structural components, cells, and so forth. Implicit, however, in the nature of an autoimmune condition is the notion that at least one autoantigen must be involved in creating the sequence of events that results in the symptoms. Autoimmune demyelinating diseases, such as multiple sclerosis (MS), are no exception. MS is the most common cause of neurological disability associated with disease in Western countries. It is an inflammatory disease of the central nervous system (CNS) characterized in part by destruction of myelin.

Macrophages, plasma cells, antigen-presenting cells, and cytokine-secreting T lymphocytes can be found in the CNS of MS patients, but activated. The etiology of MS is still unknown, but it is believed that activated T cells misdirected towards normal constituents of the nervous system are responsible for the pathology of MS. Models for MS and compositions and methods for treating MS are thus needed.

A commonly used animal model for MS is experimental allergic encephalomyelitis (EAE). EAE is a CD4+ T cell mediated autoimmune demyelinating disease of the CNS which resembles MS in some of its clinical and histological features. This disease can be induced, for example, in guinea pigs by administration of whole brain homogenate, and has been induced in mice with myelin basic protein (MBP) and complete Freund's adjuvant. The mouse model has been accepted as a model of human MS. Animals so immunized exhibit symptoms of EAE, including, but not limited to, paralysis and often death. Although MBP has been associated with EAE and MS, the search for other factors and other autoantigens which may contribute to these diseases continues (Kuby (1994) *Immunol.* (2d Ed.) pp. 451-457).

The apparent first indication of the autoantigen which came to be known as myelin oligodendrocyte glycoprotein (MOG) was disclosed by Lebar *et al.* (*J. Immunol.* (1976) **116**:1439-1446). This work reported the results of a study which identified an IgG2 antibody in the serum of EAE guinea pigs as responsible for the complement-dependent demyelinating activity of the serum. The relevant antibody reacted with the putative autoantigen present in the homogenate of CNS myelin. This autoantigen was shown to be different from the encephalitogenic MBP of CNS myelin.

In a subsequent paper, this unknown antigen, now designated M2, was identified as a relatively minor component of CNS myelin located on oligodendrocyte surfaces and in the outermost lamellae of myelin in mouse, rabbit, rat, bovine and human CNS tissues as well as guinea pig (Lebar *et al.* (1986) *J. Exp. Immunol.* **66**:423-443). M2 appeared as two glycoprotein bands at 27 and 54 kD, and monoclonal antibodies putatively specific for M2 were also reported in this paper.

In 1987, the role of M2, now called MOG, was partially elucidated (Linington et al. (1987) *J. Neuroimmunol.* (1987) **17**:61-69). *In vivo* demyelinating activity of serum in rats was correlated with its anti-MOG antibody

titer. The authors suggest that antibodies against MOG are involved in the pathogenesis of the model, in this case, chronic relapsing EAE. Further studies by this group further elucidated the relevant events in the EAE model (Lassmann et al. (1988) *Acta Neuropathol (Berl)* **75**:566-576). This study showed a relationship between anti-MOG antibody and high numbers of T-cells in determining the nature of the observed symptoms. In a still later paper, it was shown that a monoclonal antibody directed against MOG induced demyelination in aggregating brain cell cultures (Kerleio deRosbo et al. (1990) *J. Neurochem.* **55**:583-587).

Sun et al. (*J. Immunol.* (1991) **146**:1490-1495), directly studied MS in patients and evaluated both B- and T-cell response to MOG. This paper established that the T-cell response to MOG is Class II restricted and confirmed that MOG is an important antigen in MS.

Purified murine MOG has been shown to migrate as a 26-28 kD doublet band with a minor band at 53 kD on SDS-page immunoblots (Matthieu et al. (1990) *Dev. Neurosci.* **12**:293-302). Amiguet et al. (*J. Neurochem.* (1992) **58**:1676-1682), had shown that chemical and enzymatic deglycosylation of murine MOG resulted in a single 25 kD peptide. Thus, murine MOG in its monomeric form is a 25 kD amino acid sequence. This article also disclosed the first approximately 26 amino acids at the N-terminus of the murine protein. Gardinier et al. (*J. Neurosci. Res.* (1992) **33**:177-187, isolated several rat MOG cDNAs and confirmed their identity by comparison with murine MOG N-terminal peptide sequence. The rat cDNA obtained by Gardinier (*supra*) had an open reading frame that encoded a putative signal peptide of 27 amino acids followed by mature MOG peptide of 218 amino acids with a calculated molecular weight of 24.962 kD. The N-terminal amino acid sequences obtained for the murine protein were similar to those deduced from the rat cDNA.

SUMMARY OF THE INVENTION

The present invention provides recombinant materials for the production of the human MOG protein as well as the complete amino acid sequence thereof. Using this information, the MOG protein or useful peptides representing portions of the amino acid sequence of MOG protein can be determined and are useful in the diagnosis and treatment of demyelinating autoimmune diseases in humans. Further, methods of screening for, and developing therapeutics compositions for, and treating autoimmune disease are disclosed.

The invention provides the complete amino acid sequence of human MOG protein (SEQ ID NO:2) as well as recombinant materials for the production of the protein and fragments thereof. Knowledge of the amino acid sequence permits design of peptide portions thereof which are useful in diagnosis and treatment of autoimmune diseases.

Accordingly, in one aspect, the invention is directed to isolated and purified human MOG protein, and to peptide fragments thereof which modulate the course of development of symptomology in demyelinating autoimmune diseases. In one embodiment, the MOG peptides of the invention have an amino acid sequence selected from the group consisting of SEQ ID NOS:96-110 and 146-164. In another embodiment, the invention provides human MOG peptides including at least one T cell epitope-containing region and comprising all or a portion of amino acid residues 1-95 (SEQ ID NO:205), 101-135 (SEQ ID NO:207) including 111-135 (SEQ ID NO:102), or 171-215 (SEQ ID NO:206).

In yet another aspect, the invention is directed to recombinant materials and methods useful for the production of the MOG protein and peptide portions thereof. In still another aspect, the invention is directed to compositions including pharmaceutical compositions and methods of their use in mitigating the effects of demyelinating autoimmune diseases such as MS.

In another aspect, the invention is directed to isolated, purified, and modified human MOG protein and peptides in which conservative substitutions have been made which exhibit characteristics of T cell epitopes of the naturally occurring MOG protein and peptides. In one embodiment, a MOG peptide of the invention has a first amino acid substituted for a second amino acid. The peptide so modified retains a biological activity which it possessed before modification. In another embodiment, a first amino acid is substituted for a second, a third, and/or a fourth amino acid in the MOG peptide. In yet another embodiment, the peptide is coupled to a polyethylene glycol, a moiety that enhances the solubility of the peptide, a moiety that facilitates purification of the peptide, and/or a moiety including a proteolytic cleavage site.

Other embodiments include a MOG peptide of the invention including at least one T-cell epitope. In some embodiments, such a MOG peptide includes at least two T cell epitopes. In other embodiments, the MOG peptide includes tandem copies of the same or different T cell epitopes.

In yet another aspect, the invention discloses a method for screening for demyelinating autoimmune disease and identifying therapeutic compositions comprised of MOG or fragments thereof which are capable diagnosing, preventing or treating MS in mammals, preferably, humans.

The invention also provides compositions including at least one MOG peptide. In some embodiments, the MOG peptide of the composition of the invention comprises at least one T cell epitope. In other embodiments, the composition further comprises a peptide of human MBP. In some embodiments, the composition includes tandem copies of a MOG peptide.

Multipeptide compositions are also provided by the invention. These compositions include more than one MOG peptide of the invention or a combination of a MOG peptide of the invention and any other MOG peptide or peptide of human MBP.

In another aspect, such compositions and multipeptide compositions are part of therapeutic compositions, including a pharmaceutically acceptable carrier, for treating multiple sclerosis in a mammal. In other embodiments, a therapeutic composition is provided which includes a pharmaceutically acceptable excipient and a major histocompatibility complex (MHC) class II peptide complex capable of binding a T cell receptor and inducing anergy and/or apoptosis in a T cell bearing the receptor. In this embodiment, the complex includes an MHC class II component comprises extracellular domains of an MHC class II molecule sufficient to form an antigenic binding pocket, is encoded by an allele associated with an autoimmune disease such as MS, and is soluble under physiological conditions in the absence of detergent or lipids. The complex further includes a MOG peptide which is autoantigenic and bound to the antigen binding pocket. In some embodiments, the complex further includes at least a second MHC class II peptide complex. In yet oether embodiments, the therapeutic formulations of the invention include MOG or MBP + MOG, or MBP peptides.

In yet another aspect, the invention provides a method of treating MS comprising the step of administering to a mammal suffering from MS a therapeutic composition of the invention in an amount sufficient to down-regulate an autoimmune response in the mammal. In some embodiments, the administering step is carried out by intravenous injection, subcutaneous injection, intramuscular injection, oral administration, inhalation, sublingual administration, transdermal administration, or rectal administration. In a particular embodiment, the composition is administered subcutaneously in non-immunogenic form in an amount sufficient to down-regulate the autoimmune response in the mammal.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

FIG. 1 shows the complete nucleotide sequence (SEQ ID NO:1) of DNA encoding human MOG protein and the amino acid sequence deduced therefrom (SEQ ID NO:2), wherein the arrow indicates the first amino acid of the mature MOG protein;

FIG. 2 shows peptides, designated by amino acid sequence which are useful in the invention (SEQ ID NOS:4-9, 11, 15, 16, and 42-72);

FIG. 3 is a table showing the effect of each of the natural amino acids at certain positions on peptides binding to the MHC Class II product of DR4;

FIG. 4 is a table showing a comparison of predicted and measured IC_{50} values for 12 peptides (SEQ ID NOS:19-32) binding to the MHC Class II product of DR4;

FIG. 5a shows peptides (20mers) designated by amino acid sequence (SEQ ID NOS:73-93) which are useful in the invention;

FIG. 5b shows a peptide of human MOG 1-121, designated by amino acid sequence, which contains at least one T cell epitope;

FIG. 6 is a bar graph of data from Example 5 where the X-axis indicates wells containing 4 different cell lines, the 7 peptides tested are indicated by the different legends and the Y-axis indicates counts per minute where the data is expressed as ^{3}H-thymidine incorporated (CPM) by each cell line in response to each individual peptide;

FIG. 7 is a schematic representation of the identity of representative MOG peptides compared with the complete MOG sequence;

FIG. 8A is a bar graph of data from Example 8 where the x axis indicates individual MOG peptides used to test short term MOG-selected T cell lines, and the y axis indicates the sum of ranks earned by each peptide;

FIG. 8B is a bar graph of data from Example 8 where the x axis indicates individual MOG peptides used to test short term MOG-selected T cell lines, and the y axis indicates the average value for percent of total peptide reactivity accounted for by each individual peptide; and

FIG. 9 is a bar graph of data from Example 9 where the x-axis indicates individual MOG peptides used to test short term MOG-selected T cell lines, and the y axis (positivity index) indicates the average value for percent of total peptide activity accounted for by each individual peptide.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. The issued U.S. patents, allowed applications, published foreign applications, and references cited herein are hereby incorporated by reference.

The present invention provides novel peptides of human MOG, an autoantigen involved in demyelinating autoimmune diseases. It also compositions including such peptides which are useful in moderating the autoimmune response. Many of these peptides are characterized by their correspondence to the T-cell epitope regions of the human MOG.

While the etiology of development of the symptoms of an autoimmune disease such as MS far from clear, certain events are believed to be critical to the progression of the condition. Demyelinating autoimmune diseases involve a T cell mediated attack by the immune system on the myelin sheath, resulting in what amounts to short circuits in the nervous system. T-cell responses to the human MOG autoantigen require uptake and subsequent proteolytic cleavage of the antigen by antigen presenting cells, followed by presentation of the antigen in the context of a Class II major histocompatibility complex (MHC)-encoded protein, thereby permitting their recognition by the T-cells. Thus, the T-cell epitope regions of the autoantigen are those which are presented by the Class II MHC proteins to the T-cell receptors. The relevant T-cells can be rendered nonresponsive in an antigen-specific fashion in protocols by providing the T-cell epitope regions of the autoantigen, as is further described below.

A nucleic acid sequence encoding human MOG is shown in FIG. 1 (SEQ ID NO:1), along with the deduced amino acid sequence (SEQ ID NO:2). The encoded mature protein contains a 218 amino acids; the full length protein (including signal peptide) is 87% homologous with the rat MOG protein. As described above, the availability of the deduced amino acid sequence of human

MOG provides the opportunity to design peptide fragments thereof which induce immune responses in mammals and peptide fragments which are T-cell epitopes, i.e., constitute those portions of the molecule which are recognized by human T-cell receptors. These peptides and fragments are also included within the invention scope of the invention.

However, the scope of the invention is not limited to the human MOG protein encoded by the amino acid sequence depicted in FIG. 1, or to the specific nucleic acid sequence presented. Naturally occurring variants and deliberate mutations designed to modify the nucleic acid sequence *per se* or to modify the encoded protein are also included in the scope of the invention as further described below. With respect to naturally occurring variants, DNA sequence polymorphisms, especially those resulting in "silent" mutations which do not affect the amino acid sequence of the human MOG, but also sequence polymorphisms that do lead to changes in the amino acid sequence, are expected to exist in the human population. These variations in one or more nucleotides (up to about 1% of the nucleotides) of the sequence encoding MOG are a result of natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of the invention. Furthermore, there may be one or more "family members" of MOG that are related in function and amino acid sequence to the MOG encoded by the DNA disclosed herein but encoded by separate genes. Such family members are also included within the definition of human MOG and the nucleotide sequences encoding it.

Isolated autoantigenic proteins or fragments thereof, that are novel and that are immunologically related to human MOG or fragments thereof, other than those already identified, are within the scope of the invention. These can be identified by antibody cross-reactivity or T-cell cross-reactivity. Such proteins or fragments thereof bind antibodies specific for the protein and peptides of the invention, or stimulate T-cells specific for the protein and peptides of this invention.

"Antigenic fragments" refer to an amino acid sequence having fewer amino acid residues than the entire protein and include fragments or peptides which induce an immune response in mammals, preferably humans, such as eliciting the production of IgG and IgM antibodies, or eliciting a T-cell response such as proliferation and/or lymphokine secretion and/or induction of T-cell anergy and/or apoptosis and/or modification of TH_1 and TH_2 subsets. Of particular interest are antigenic fragments that comprise T-cell epitopes. Peptides can be derived from the naturally occurring MOG sequence or and can be modified such that conservative amino acid substitutions have been made. These substitutions include replacing another amino acid (e.g., a second, third, fourth, or other amino acid), replacing more than one amino acid (e.g., a second and third, second, third and fourth, or other amino acids) with a first amino acid which does not interfere with a biological activity of the peptide; i.e., The peptide so modified retains a biological activity it possessed before modification. Nonlimiting examples of naturally occurring and modified peptides are shown in FIG. 2 (SEQ ID NOS:4-9, 11, 15, 16, 42-72. and 169), and in Tables 1 and 2 below.

TABLE 1

Peptide	Sequence (NH2 → COOH)	SEQ ID NO:
MOG 1-13	GQFRVIGPRHPIR	42
MOG 20-32	VELPCRISPGKNA	5
MOG 70-82, A78	ELLKDAIGAGKVT (analog of MOG 70-82)	8
MOG 88-100,K89,S98	VKFSDEGGFTSFF (analog of MOG 88-100)	9
MOG 103-115	HSYQEEAAMELKV	55
MOG 118-130	PFYWVSPGVLVLL	56
MOG 170-182	PHRLRVPCWKITL	15
MOG 141-160	TVGLVFLCLQYRLRGKLRAE	209
MOG 199-218	YNWLHRRLAGQFLEELRNPF	210

TABLE 2

peptide	Sequence (NH2 → COOH)	SEQ ID NO:
1-20	GQFRVIGPRHPIRALVGDEV	220
1-25	GQFRVIGPRHPIRALVGDEVELPCR	109
1-22	GQFRVIGPRHPIRALVGDEVEL	146
1-25 (24C-24S)	GQFRVIGPRHPIRALVGDEVELPSR	147
11-35	PIRALVGDEVELPCRISPGKNATGM	148
21-40	ELPCRISPGKNATGMEVGWY	221
21-45	ELPCRISPGKNATGMEVGWYRPPFS	110
31-55	NATGMEVGWYRPPFSRVVHLYRNGK	96
41-60	RPPFSRVVHLYRNGKDQDGD	222
41-65	RPPFSRVVHLYRNGKDQDGDQAPEY	97
36-60	EVGWYRPPFSRVVHLYRNGKDQDGD	149
46-70	RVVHLYRNGKDQDGDQAPEYRGRTE	150
51-75	YRNGKDQDGDQAPEYRGRTELLKDA	98
61-85	QAPEYRGRTELLKDAIGEGKVTLRI	99
71-95	LLKDAIGEGKVTLRIRNVRFSDEGG	100
81-100	VTLRIRNVRFSDEGGFTCFF	151
81-105	VTLRIRNVRFSDEGGFTCFFRDHSY	152
91-110	SDEGGFTCFFRDHSYQEEAA	223
91-115	SDEGGFTCFFRDHSYQEEAAMELKV	153
101-125	RDHSYQEEAAMELKVEDPFYWVSPG	101
111-130	MELKVEDPFYWVSPGVLVLL	154
111-135	MELKVEDPFYWVSPGVLVLLAVLPV	102
121-140	WVSPGVLVLLAVLPVLLLQI	155
121-145	WVSPGVLVLLAVLPVLLLQITVGLV	156
131-150	AVLPVLLLQITVGLVFLCLQ	157
131-155	AVLPVLLLQITVGLVFLCLQYRLRG	103
141-165	TVGLVFLCLQYRLRGKLRAEIENLH	158
151-175	YRLRGKLRAEIENLHRTFDPHFLRV	105
161-185	IENLHRTFDPHFLRVPCWKITLFVI	106
171-190	HFLRVPCWKITLFVIVPVLG	159
171-195	HFLRVPCWKITLFVIVPVLGPLVAL	107
171-185	HFLRVPCWKITLFVI	160
181-195	TLFVIVPVLGPLVAL	161
176-190	PCWKITLFVIVPVLG	162
181-200	TLFVIVPVLGPLVALIICYN	163
181-205	TLFVIVPVLGPLVALIICYNWLHRR	164
191-210	PLVALIICYNWLHRRLAGQF	104
191-215	PLVALIICYNWLHRRLAGQFLEELR	108

Preparation of Nucleic Acids

Nucleic acid molecules containing a sequence encoding human MOG or an antigenic fragment thereof may be obtained by reverse transcription of mRNA present in human brain or other CNS tissue, as well as from genomic DNA.

Various methods of chemically synthesizing polynucleotides are known, including standard solid-phase synthesis which, like peptide synthesis, has been fully automated in commercially available DNA synthesizers (See e.g., Itakura *et al.*, U.S. Patent No. 4,598,049; Caruthers *et al.*, U.S. Patent No. 4,458,066; and Itakura, U.S. Patent Nos. 4,401,796 and 4,373,071). The nucleic acid molecules of the invention also include RNA which can be transcribed from the DNA prepared as above or synthesized chemically.

Preparation of Human MOG and its Fragments

The present invention also provides expression systems and host cells transformed with these systems for production of the encoded protein. Host cells include bacterial cells such as *E. coli*, yeast, or mammalian cells such as Chinese hamster ovary cells (CHO). Suitable host cells and expression vectors containing relevant promoters, enhancers and other expression control elements may be found in Goeddel, *Gene Expression Technology: Methods in Enzymology* Vol. 185, Academic Press, San Diego, California (1990). Other suitable host cells and expression vectors are known to those skilled in the art.

Expression in eucaryotic cells such as mammalian, yeast, or insect cells can lead to partial or complete glycosylation and/or formation of relevant inter- or intra-chain disulfide bonds of recombinant protein. Examples of vectors for expression in yeast *S. cerevisae* include pYepSec1 (Baldari. *et al.*, (1987) *Embo J.* **6**:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* **30**:933-943), pJRY88 (Schultz *et al.* (1987) *Gene* **54**:113-123), and pYES2 (Invitrogen Corp., San Diego, CA). Baculovirus vectors available for expression of proteins in cultured insect cells (Sf9 cells) include the pAc series (Smith et al. (1983) *Mol. Cell Biol.* **3**:2156-2165) and the pVL series (Lucklow et al. (1989) *Virol.* **170**:31-39). Generally, COS cells (Gluzman (1981) *Cell* **23**:175-182) are used in conjunction with such vectors as pCDM 8 (Aruffo et al. (1987) *Proc. Natl. Acad. Sci. (USA)* **84**:8573-8577) for transient amplification/expression in mammalian cells, while CHO-DHFR cells are used with vectors such as pMT2PC (Kaufman *et al.* (1987),

EMBO J. **6**:187-195) for stable amplification/expression in mammalian cells. Vector DNA can be introduced into mammalian cells via any known conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, or electroporation. Suitable methods for transforming host cells can be found in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press (1989)), and other laboratory textbooks.

Although those skilled in the art use various methods of expression, expression in prokaryotes is most often carried out in *E. coli* with either fusion or non-fusion inducible expression vectors. Fusion vectors usually add a number of amino terminal amino acids to the expressed target gene. These amino terminal amino acids often are referred to as a reporter group. Such reporter groups usually serve two purposes: 1) to increase the solubility of the target recombinant protein; and 2) to aid in the purification of the target recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the reporter group and the target recombinant protein to enable separation of the target recombinant protein from the reporter group subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase, maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Inducible non-fusion expression vectors include pTrc (Amann *et al.*, (1988) *Gene* **69**:301-315) and pET 11d (Studier et al. (1990) *Meth. Enzymol.* **185:**60-89). While target gene expression relies on host RNA polymerase transcription from the hybrid Trp-lac fusion promoter in pTrc, expression of target genes inserted into pET 11d relies on transcription from the T7 gn10-lac 0 fusion promoter mediated by coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident g

prophage harboring a T7 gn1 under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman (1990) *Meth. Enzymol.* **185**:119-128). Another strategy is to alter the coding sequence of the gene so that the individual codons for each amino acid are those preferentially utilized in highly expressed *E. coli* proteins (Wada et al. (1992) *Nucleic Acids Res.* **20**:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

Upon expression of the encoding gene, the recombinant protein, or peptide product may be secreted and harvested from the medium. Alternatively, the protein may be retained cytoplasmically and the cells harvested, lysed, and the protein isolated and purified. Suitable media for cell culture are well known in the art. The protein and peptides of the invention can be purified from cell culture medium, host cells, or both using techniques known in the art for purifying proteins and peptides including ion-exchange chromatography, gel filtration chromatography, metal affinity chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with specific antibodies. The terms isolated and purified are used interchangeably herein and refer to peptides, protein, protein fragments, and nucleic acid molecules substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Accordingly, an isolated peptide is produced recombinantly or synthetically and is substantially free of cellular material and culture medium or substantially free of chemical precursors or other chemicals.

Antigenic Fragments and the "Antigenic" Response

Fragments of the MOG protein that elicit a desired antigenic response (referred to interchangeably herein as antigenic fragments or peptides) may be obtained, for example, by screening peptides corresponding to portions of the protein. These peptides may be chemically synthesized using techniques known in the art, produced recombinantly, or prepared through proteolysis of the whole or portion of the protein. For example, the protein may be arbitrarily divided into fragments of desired length with no overlap of the fragments, or preferably divided into overlapping fragments of a desired length (see, e.g., FIG. 7). The fragments are tested to determine their antigenicity (e.g., the ability of the fragment to induce an immune response in a mammal). If fragments of the protein are to be used for therapeutic purposes, then the fragments which are capable of eliciting a T-cell response, such as stimulation (i.e., proliferation or lymphokine secretion) and/or are capable of inducing T-cell tolerance, anergy, and/or apoptosis are particularly desirable.

The isolated protein or preferred antigenic fragments thereof, when administered to an individual subject to demyelinating autoimmune disease, are capable of modifying the B-cell response, T-cell response, or both the B-cell and the T-cell response of the individual to the autoantigen, or can be shown to result in a diminution of symptoms. As referred to herein, "a diminution in symptoms" includes any reduction in inflammation or in infiltration of leukocytes and/or arrest of demyelination characteristic of the disease condition following a treatment regimen with a peptide or protein of the invention. This diminution in symptoms may be determined subjectively or clinically.

Human T-cell stimulating activity can be tested by culturing T-cells obtained from a subject having an autoimmune condition with the autoantigen and/or a peptide derived from the autoantigen and determining whether proliferation of T-cells occurs in response to the autoantigen and/or peptide as measured, e.g., by cellular uptake of tritiated thymidine. Stimulation indices for

responses by T-cells to peptides can be calculated as the maximum counts per minute (CPM) in response to a peptide divided by the control CPM. A stimulation index (S.I.) equal to or greater than two times the background level is considered "positive". Positive results are used to calculate the mean stimulation index for each peptide for the group of patients tested. Preferred peptides of this invention comprise at least one T-cell epitope and have a mean T-cell stimulation index of greater than or equal to 1.5. A peptide having a mean T-cell stimulation index of greater than or equal to 1.5 in a significant number of patients tested (i.e. at least 10% of patients tested) is considered useful as a therapeutic agent. Preferred peptides have a mean T-cell stimulation index of at least 1.5, more preferably at least 2.0 to 3.0.

Preferred peptides can also be identified by their ability to effect a relatively higher frequency of T-cells in a patient. This frequency is measured by generating multiple identical cultures from one patient with limiting numbers of lymphocytes and the autoantigen and/or a peptide from the autoantigen. Individual cultures are analyzed for positive reactivity with a peptide, as defined by stimulation index (described above). The frequency of peptide-reactive T-cells is the percentage of cultures from the patient that show a positive stimulation index.

In addition, preferred peptides have a positivity index (P.I.) of at least about 100, more preferably at least about 200 and most preferably at least about 300. The positivity index for a peptide is determined by multiplying the mean T-cell stimulation index by the percent of individuals, in a population of autoimmune patients (e.g., preferably at least 15 individuals, more preferably at least 30 individuals or more), who have a T-cell stimulation index to such peptide of at least 1.5, more preferably at least 2.0. Thus, the positivity index represents both the strength of a T-cell response to a peptide (S.I.) and the frequency of a T-cell response to a peptide in a population of autoimmune individuals.

In order to determine precise T-cell epitopes by, for example, fine mapping techniques, a peptide having T-cell stimulating activity and thus comprising at least

one T-cell epitope as determined by T-cell biology techniques is modified by addition or deletion of amino acid residues at either the amino or carboxy terminus of the peptide and tested to determine a change in T-cell reactivity to the modified peptide. If two or more peptides which share an area of overlap in the native protein sequence are found to have human T-cell stimulating activity, as determined by T-cell biology techniques, additional peptides can be produced comprising all or a portion of such peptides and these additional peptides can be tested by a similar procedure. Following this technique, peptides are selected and produced recombinantly or synthetically. Peptides are selected based on various factors, including the strength of the T-cell response to the peptide (e.g., stimulation index) and the frequency of the T-cell response to the peptide in a population of autoimmune subjects. The physical and chemical properties of these selected peptides (e.g., solubility, stability) are examined to determine whether the peptides are suitable for use in therapeutic compositions or whether the peptides require modification as described herein. The ability of the selected peptides or selected modified peptides to stimulate human T-cells (e.g., induce proliferation, lymphokine secretion) is determined.

A T-cell epitope-containing peptide of the invention, when administered to a subject in a therapeutic treatment regimen is capable of modifying the response of the individual to the autoantigen.

Preferred peptides of the invention comprise at least one T-cell epitope of the full length protein. Accordingly the peptide comprises at least approximately 7, preferably at least about 12-40, and more preferably 13-30 amino acid residues. The peptides may contain tandem repeats of a single epitope and/or more than one different epitope. In other aspects, compositions of the invention contain tandem copies of MOG peptides. For purposes of therapeutic effectiveness, preferred therapeutic compositions of the invention preferably comprise at least two T-cell epitopes. Additionally, therapeutic compositions comprising one or more preferred isolated peptides of the invention preferably comprise a sufficient percentage of the T-cell epitopes of the entire protein such that a therapeutic

regimen of administration of the composition results in amelioration of disease symptoms. Synthetically produced peptides of the invention comprising less than approximately 45 amino acid residues, and most preferably less than approximately 30 amino acid residues are particularly desirable for ease of peptide synthesis. Peptides of the invention may also be produced recombinantly as described above. Preferable, peptides of 45 amino acids or longer greater are produced recombinantly.

Isolated antigenic peptide fragments which have T-cell stimulating activity, and thus comprise at least one T-cell epitope are particularly desirable. An "epitope" is the basic element, or smallest unit of recognition by a receptor, particularly immunoglobulins, histocompatibility antigens, and T-cell receptors, where the epitope comprises amino acids of the native protein. Amino acid sequences which mimic those of the epitopes can also be used. A "T-cell epitope" is the basic element, or smallest unit of recognition by a T-cell receptor, where the epitope comprises amino acids in the autoantigen essential to receptor recognition. Amino acid sequences which mimic those of the native T-cell epitopes are also within the scope of this invention. T-cell epitopes are believed to be involved in initiation and perpetuation of the autoimmune response. These T-cell epitopes are thought to trigger early events at the level of the T helper cell by being presented by an appropriate HLA molecule on the surface of an antigen presenting cell, thereby stimulating the T-cell subpopulation with the relevant T-cell receptor for the epitope. These events lead to T-cell proliferation, lymphokine secretion, local inflammatory reactions, recruitment of additional immune cells to the site of antigen/T-cell interaction, and activation of the B-cell cascade leading to the production of antibodies.

Exposure of a subject to a peptide or protein which comprises at least one T-cell epitope of the autoantigen may tolerize, anergize, induce apoptosis, or otherwise modify appropriate T-cell subpopulations such that they become non-responsive to the autoantigen and do not participate in stimulating an immune response.

As used herein, to "tolerize" is defined as to induce a state of non-responsiveness to subsequent challenge with antigen by any of a variety of mechanisms, including, but not limited to, clonal deletion or apoptosis of antigen-specific T cells, prevention of appropriate presentation of the antigen by relevant antigen presenting cells, or induction of immune cells with suppressive, killer or anti-inflammatory capabilities, having specificity for the antigen itself or for the relevant antigen-specific T cells. The term "angergize" refers to the induction of a state of non-responsiveness to subsequent challenge with an antigen, despite the continued presence of the T cells bearing the relevant antigen-specific receptors. The term "apoptosis" refers to the programmed cell death characterized by degradation of the cell nuclear structure, mediated by intracellular nucleases, and initiated by any of a variety of extra-cellular signals, including those related to activation of the antigen-specific T cell.

In addition, administration of a protein or peptide which comprises at least one T-cell epitope may modify the lymphokine secretion profile as compared with exposure to the naturally-occurring autoantigen (e.g., result in a decrease of IL-4 and/or an increase in IL-2 causing a modification of TH_1 and TH_2 populations). Furthermore, exposure to such a protein or peptide may influence T-cell subpopulations which normally participate in the response to the autoantigen such that these T-cells are drawn away from the site(s) of normal exposure to the autoantigen (e.g., tissues of the CNS) to the site(s) of therapeutic administration of the protein or peptide derived therefrom. This redistribution of T-cell subpopulations may ameliorate or reduce the ability of an individual's immune system to stimulate the usual immune response at the site of normal exposure to the autoantigen, resulting in a diminution in symptoms.

In determining which peptides are T cell epitope-containing peptides for a specific disease, those skilled in the art can approach the task in many acceptable ways. In the instant invention, the procedures selected are not meant to be limiting. The selection of one mode of approaching a task is not intended to exclude other modes which can accomplish the same end by alternative means by

those skilled in the art, for example, the selection of likely T cell epitope-containing peptide from a group of peptides. Generally, in the instant application, as further explained in more detail herein, a protein is purified and analyzed, peptides are selected for testing, the selected peptides are produced, and the selected peptides are tested for properties characteristic of T cell epitopes.

T-Cell Epitope Regions of Human MOG

Peptides derived from human MOG which moderate the response of a subject to the MOG autoantigen are also included in the invention. Likely candidates for such peptides can be tested for the effect on T cell proliferation, as previously discussed and/or testing for a candidate's affinity for binding HLA DR proteins (procedures for obtaining HLA DR proteins is discussed in detail in the section below entitled "Purification and Analysis of HLA DR proteins") . Such peptides can be identified, for example, by examining the structure and selecting appropriate regions to be produced as peptides (via recombinant expression systems, synthetically or otherwise) to be examined for ability to influence B-cell and/or T-cell responses, and selecting peptides containing epitopes recognized by these cells. One method of identifying such peptides includes dividing the human MOG protein antigen into non-overlapping, or overlapping peptides of desired lengths and synthesizing, purifying and testing those peptides to determine whether the peptides comprise at least one T cell epitope using any number of assays. In another method, an algorithm is used for predicting those peptides. Other methods known to those skilled in the art may also be employed. In the instant application, certain of the peptides were selected using the algorithm as discussed in more detail herein.

A. Defining MOG Epitopes Using an MHC Binding Algorithm

Thirty-seven naturally occurring 13mers (together with three analogs of the naturally occurring peptides) are shown in FIG. 2 (SEQ ID NOS:4-9, 11, 15, 16, 42-72, and 169); the amino acid length is arbitrarily chosen. These peptides were

chosen according to an algorithm that predicts optimal MHC class II binding. In order for the peptide to be able to bind an appropriate T cell receptor, it is necessary that it be able to bind class II MHC proteins. It is possible to remove at least one or two amino acids from the N- and/or C-terminus of each and still retain MHC binding activity. These peptides are designated by amino acid sequence and represent the indicated regions of the mature amino acid sequence shown as positions 1-218 of FIG. 1 (SEQ ID NO:2). The peptides are characterized by their ability to bind Class II MHC proteins so as to have the ability to be presented effectively as T-cell epitopes.(Rothbard et al. (1988) *EMBO J.* 7:93-100). It has been found that a necessary (but not necessarily sufficient) condition for binding to Class II MHC proteins is the presence of a hydrophobic side chain residue, most preferably a tyrosine, phenylalanine or tryptophan residue, and preferably isoleucine, leucine, valine or methionine residue spaced at a four amino acid distance from a small amino acid residue such as glycine, alanine, serine, threonine or cysteine. All of the MOG peptides shown in FIG. 2 (SEQ ID NOS:4-9, 11, 15, 16, 42-72, and 169) fulfill these minimum conditions. As further shown in FIG. 2, all of these peptides have been tested with respect to their ability to bind MHC proteins encoded by certain alleles. As shown by the IC_{50} values in the figure, most of these peptides bind tightly to both DRB1*0101 and DR B1*1501. The specifics of peptide binding are described below. FIG. 2 is intended to be a non-limiting, representative example of the algorithm for selection. However, other possible useful antigenic peptides may exist.

The requirement for the four amino acid spacing between the hydrophobic residue and small residue used as the basis for identifying the peptides as described in the preceding paragraph has been verified using experimental protocols as follows: The design of the experiments assumed that all peptides bind in approximately identical locations, oriented by interactions with the peptide backbone and adopt closely related conformations. The design of the experiments also assumed that the binding site of MHC Class II molecules can be divided into separate subsites that differentially contribute to binding, that the overall free energy of binding is the sum of the advantageous and deleterious contacts a

peptide makes with the pockets, and that the interaction between the peptide in each of the pockets can be viewed independently. The results of the experiments support these postulates, as well as a model of free energy of binding as a simple polynomial with separate terms for backbone interactions in the side chains. For peptides of common length, the backbone terms would be constant while the contribution of side chains would vary depending on their structure and the chemical composition and size of the complementary pocket in the binding site. The relative importance of each side chain position will vary depending on the allele and the isotype, but in general peptide binding can be viewed as the sum of separate independent events.

Thus, the results of applicants' experiments show that the apparent affinity of any sequence of common length can be predicted within a factor of two or three of the experimentally determined values based on a database of the relative effects of the natural amino acids in each position of model peptides. All possible monosubstituted analogs at the central 11 positions of the simplified peptide, AAYAAAKAAAAAA (SEQ ID NO:33) were synthesized and assayed for binding to DRB1*0401 (formerly called DR4DW4). From the measured IC_{50} values of each analog, a ratio relative to the parent simplified peptide was calculated. These ratios and the measured values are shown in FIG. 3. The ratios were then used to predict the affinity of 12 unrelated natural sequences corresponding to peptides known to bind DRB1*0401 with a wide range of affinity as shown in FIG. 4 (SEQ ID NOS:19-32). The data shown in FIGS. 3 and 4 verify that the model is useful in predicting the four amino acid spacing and is consistent with experimental results. Using this model, candidates of 13mers were selected as likely T cell epitope-containing peptides.

The procedures used for detergent solubilization and affinity purification of HLA-DR (human MHC protein) molecules were similar to those described by Gorga (*J. Biol. Chem.* (1987) **262**:16087-16094) and Buelow (*Eur. J. Immunol* (1993) **23**:69-76). Briefly, CHO cells transfected with the genes for the DRB1*0101 and DRB1*1501.alleles (Marshall et al. (1994) *J. Immunol.* **152**:4946-

4957) were grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum (FCS), 2 mM glutamine, and antibiotics. Cells were harvested, washed in PBS, lysed with 1% NP-40, and the supernatant separated from nuclear debris by centrifugation. The solubilized MHC class II proteins were affinity purified using the monoclonal antibody LB3.1, coupled to Sepharose CL4B. Class II proteins were eluted with 1% octyl-β-D-glucopyranoside (octyl glucoside), 50 mM phosphate pH 11.5 and immediately neutralized using 1 M phosphate pH 6.0. Purified $\alpha\beta$ heterodimers were isolated by size exclusion using a 60 cm x 2.5 cm diameter column of Bio-Gel A0.5. The fractions containing the heterodimers were concentrated using Amicon Centri-Prep® 30 devices to a nominal concentration of 500 μg/ml.

The purity of the material was assayed by SDS-PAGE, high performance size exclusion chromatography (HPSEC), and Edman sequencing as previously described (Buelow et al., 1993). The HPSEC column was a BIOSEP SEC-S3000 (300 x 7.5 mm) (Phenomenex), eluted using a buffer system of PBS containing 1.0% octyl glucoside and 1.0% acetonitrile at a flow rate of 0.800 ml/min (approximately 25 minutes per run). The fluorescence detector was set to monitor tryptophan fluorescence (λ_{ex} = 282 nm, λ_{em} = 348 nm).

Peptide binding assays were performed as previously described (Hill et al. (1994) *J. Immunol.* **152**:2890-2898). Briefly, affinity purified class II proteins (10 nM) were incubated with serial dilutions of the test peptide and a fixed concentration of biotinylated HA 307-319 (2 nM) in PBS containing 1.0% octyl glucoside at pH 6.5 in 96 well polypropylene plates (Costar, Cambridge, MA) for 16 hours at 37°C. The DR-peptide complexes (50 μl) were transferred, in duplicate, to wells of a 96-well microtiter plate precoated with the monoclonal antibody LB3.1 and blocked with fetal calf serum. Excess peptide was removed by washing with PBS containing 0.02% Tween 20 and 0.05% NaN_3. Europium-labeled streptavidin (Pharmacia, Piscataway, NJ) was added and incubated overnight. After washing, a solution of 0.1 M acetate/phthalate buffer, pH 3.2, containing 0.1% Triton X-100, 15 μM 2-naphthoyltrifluoroacetone and 50 μM tri-

N-octylphosphine oxide was added to release the chelated europium from strepavidin. The resulting fluorescence (which was proportional to the amount of bound biotinylated HA 307-319) was measured using a fluorescent plate reader (DELPHIA LKB/Pharmacia, Piscataway, NJ). The data were analyzed by fitting the data to a binding function that calculated the concentration of test peptide to bind to a specific class II protein can be ranked by the corresponding IC_{50} values, with the lower values corresponding to better binding peptides.

T cell assays were performed to further refine the identification of T cell epitopes. Peripheral blood lymphocytes were isolated from the blood of a human volunteer HLA-DR2 positive donor using standard techniques outlined in Example 2 below. The MOG peptides selected for testing were MOG 1-13 (SEQ ID NO:42), MOG 20-32 (SEQ ID NO:5), MOG 70-82, A78 (analog to MOG 70-82) (SEQ ID NO:8), MOG 88-100,K89,S98 (analog to MOG 88-100) (SEQ ID NO:9), MOG 103-115 (SEQ ID NO:55), MOG 118-130 (SEQ ID NO:56), and MOG 170-182 (SEQ ID NO:15).

Results of one such study of T cell response to human MOG peptides are illustrated in FIG. 6. These results indicate that MOG 1-13 (SEQ ID NO:42) is a T cell epitope-containing peptide. Furthermore, the same data strongly indicates that MOG 103-115 (SEQ ID NO:55) contains at least one epitope. These results do not preclude the possibility that less than the entire 13mer peptide could be a T cell epitope-containing peptide.

B. Defining MOG Peptide Epitopes Using an Overlapping Peptide Set

A second method of identifying peptides that constitute T cell epitope regions of human MOG involves dividing the human MOG protein sequence into a series of non-overlapping or overlapping peptides of desired lengths and synthesizing, purifying, and testing those peptides to determine whether the peptides comprise at least one T cell epitope using any number of assays of T cell reactivity. Using this method, a series of peptides was prepared having equal

length (e.g., 20mers or 25mers) and spanning the entire length of the MOG protein sequence while overlapping the previous peptide by 10 or 15 amino acid residues. The peptides were synthesized using standard solid-phase technology and FastMOC™ chemistry.

Testing of the peptide sets for identification of T cell epitopes was performed by two different methods. In the first, 2 x 10^5 T cells were plated into individual microtiter wells stimulated with the N-terminal fragment of recombinant human MOG or with various mixtures of the peptides. The recombinant MOG contained residues 1-121 of the MOG sequence and was purified from the supernatant of insect cells infected with the MOG expression vector construct as detailed in Example 2. The stimulated cultures were supplemented with the cytokines IL2 and IL4 to promote expansion of activated clones. After approximately two weeks, the cells in each individual microtiter culture were washed and split into four to twelve wells of a new assay plate for a second stimulation or challenge with various controls and test antigens. The proliferative response to the second antigen challenge was assessed by incorporation of tritiated thymidine.

Results from these studies indicate that MS patients have T cell responses to epitopes in the N-terminal half of the MOG protein, and that these epitopes can be encoded in peptides 20 or fewer amino acids in length. The results of one such study are detailed in Example 6A. Furthermore, the presence of an epitope in the first 25 amino acid of the MOG protein is confirmed by the application of this assay to a set of 21 MS patients. This peptide induced responses in 45.5% of the 11 MOG-responding patients in which it was tested. In addition, peptides 1-25 (SEQ ID NO:109), 41-65 (SEQ ID NO:97), and 71-95 (SEQ ID NO:100) all induced a particularly high frequency of specific response, with greater than 7% of all MOG reactive lines responding to each of these peptides. These studies are detailed in Example 6B.

In the second method of testing the peptide sets for identification of T cell epitopes, short-term MOG-reactive T cell lines were initiated from "bulk" cultures of 10 x 10^6 cells in a large tissue culture well. The antigen stimulation and cytokine expansion steps were similar to those employed in the microwell assay above, except that these cultures were all initiated by stimulation with the full length MOG protein purified from human brain tissue as detailed in Example 7. Because the starting cell number is large, the cells from a single culture can be washed after approximately two weeks of culture and split into over 100 wells of a microtiter assay plate for a second stimulation or challenge with various controls and test antigens. The proliferative response to the second antigen challenge was assessed by incorporation of tritiated thymidine, as above.

Stimulation of the initial culture with the full length protein enables testing for reactivity to a peptide set spanning the entire MOG sequence. Such tests are described in Examples 8 and 9 below. The peptides used in these studies are illustrated in FIG. 7, and are 20 or 25 amino acids in length, with overlaps of 5, 10 or 15 amino acids. The predominance of epitopes in the N-terminal half of the MOG protein was confirmed in a set of 84 MS patients. The data are illustrated in FIGS. 8A, 8B, and 9 and demonstrate that three regions are major targets of T cell response to MOG: regions including amino acid residues 1-95 (SEQ ID NO:205) including a region encompassing amino acids 1-65 (SEQ ID NO:208), 101-135 (SEQ ID NO:207) including a region encompassing amino acids 111-135 (SEQ ID NO:102), and 171-215 (SEQ ID NO:206). By several methods of data analysis, peptides 1-20 and 41-65 are confirmed as major targets of the T cell response to MOG. The peptides flanking these two sequences (11-35, 31-55, 51-75, 61-85, and 71-95) also induce significant reactivity, which may be directed at the same epitopes contained in 1-20 and 41-65, or may be due to additional epitopes that either overlap with or are completely separate from the epitopes contained in 1-20 and 41-65. Shifting, or expanding the sequences in peptides 1-20 and 41-65 may result in even greater T cell reactivity. In addition, the presence of other epitopes, peptides 101-125, 111-135, 121-145, and 131-155, 171-195, and 191-215 (or region 171-215) in the carboxy terminal half of the

protein, are also evident. Unlike all epitopes in the N-terminal half of the protein, 171-195 is predicted to lie in a transmembrane portion of the MOG molecule according to the structure predicted by Gardinier et al. (*J. Neurosci. Res.* (1992) **33**:177-187). Thus, these data demonstrate that three regions are major targets of T cell response to MOG: a region including amino acid residues 1-95 (SEQ ID NO:205), and even more particularly, amino acid residues 1-65 (SEQ ID NO:208); a region encompassing amino acids 101-135 (SEQ ID NO:207) including in particular amino acids 111-135 (SEQ ID NO:102); and a region encompassing amino acids 171-215 (SEQ ID NO:206), including in particular amino acids 171-195 (SEQ ID NO:107).

Modification of MOG Peptides

The structure of the protein or peptides of the invention can be modified for such purposes as increasing solubility, enhancing therapeutic or prophylactic efficacy, or stability (e.g., shelf life *ex vivo* and resistance to proteolytic degradation *in vivo*), or generally by conservative substitutions and modifications. A modified protein or peptide can be produced in which the amino acid sequence has been altered, such as by amino acid substitution, deletion, or addition, to modify immunogenicity, or to which a component has been added for the same purpose. FIG. 2 shows three such peptides, Human MOG 70-82, A78 (SEQ ID NO:8); Human MOG 74-86,A78 (SEQ ID NO:53); and Human MOG 88-100, K89, S98 (SEQ ID NO:9). Modification must be made in such a way that the ability to recognize the appropriate T-cell subset is not altered. It is generally understood in the art which locations of amino acid side chains are oriented toward the upper surface of the APC and which point inward. Those that point inward are suitable candidates for modification since they are less likely to affect binding to the appropriate T-cell receptor.

One embodiment of the present invention features a peptide which comprises at least one T-cell epitope of the protein and includes the regions of the peptides as shown in FIGS. 2, 5a, 5b, and/or 7 that are significant for T-cell

receptor binding or the modified forms thereof as above described, optionally extended at the N- and/or C-terminus with irrelevant amino acid sequence.

Another embodiment of the present invention provides peptides comprising at least two T-cell epitopes as described above. The T-cell epitopes may be identical or may be different T-cell epitopes appropriate for human MOG. As further described above, the T-cell epitopes, or peptides including such T cell epitopes, are typically at least seven amino acids, preferably 12-40 amino acids, even more preferably 13-30 amino acids in length. If desired, the amino acid sequences of the T-cell epitopes can be joined by a linker to increase sensitivity to processing by antigen-presenting cells. Such linker can be any non-epitope amino acid sequence or other appropriate linking or joining agent. In preferred peptides comprising at least two T-cell epitopes, the epitopes are arranged in the same or a different configuration from a naturally-occurring configuration of the epitopes in the native human MOG protein. For example, the T-cell epitope(s) can be arranged in a contiguous or noncontiguous configuration. Noncontiguous is defined as an arrangement of T-cell epitope(s) which contains additional residues between the epitopes. Furthermore, the T-cell epitopes can be arranged in a nonsequential order (e.g., in an order different from the order of the amino acids of the native protein from which T-cell epitope(s) are derived). A peptide of the invention can comprise at least 10%, at least 20%, at least 30%, at least 40%, at least 60% or more of the T-cell epitopes of human MOG. Some preferred peptides of the invention comprise various combinations of two or more of the above-discussed T-cell epitopes. Preferred peptides comprising a combination of two or more epitopes are those wherein the peptides include sequences selected from those in FIG. 2 (SEQ ID NOS:4-9, 11, 15, 16, 42-72, and 169), FIG. 5a, FIG. 5b, and FIG. 7.

A modified protein or peptide of the invention maintains its ability to induce T-cell unresponsiveness and bind MHC proteins without the ability to induce a strong or any proliferative response when administered in immunogenic form. In this instance, critical binding residues for T-cell receptor function can be

determined using known techniques (e.g., substitution of each residue and determination of the presence or absence of T-cell reactivity). Those residues shown to be essential to interact with the T-cell receptor can be modified by replacing the essential amino acid with another, preferably similar amino acid residue (a conservative substitution) whose presence is shown to enhance or diminish, but not eliminate T-cell reactivity. In addition, those amino acid residues which are not essential for T-cell receptor interaction can be modified by being replaced by another amino acid whose incorporation may enhance or diminish T-cell reactivity, but does not eliminate binding to relevant MHC.

Additionally, peptides of the invention can be modified by replacing an amino acid shown to be essential to interact with the MHC protein complex with another, preferably similar amino acid residue (conservative substitution) whose presence is shown to enhance or diminish, but not to eliminate T-cell activity. It is believed that peptides that bind MHC with higher affinity should render T-cells immunopassive *in vivo* at lower concentrations. In addition, amino acid residues which are not essential for interaction with the MHC protein complex but which are present on the bound peptide can be modified by being replaced by another amino acid whose incorporation may enhance or diminish, but not eliminate T-cell reactivity. Preferred amino acid substitutions for non-essential amino acids include, but are not limited to substitutions with alanine, glutamic acid, or a methyl amino acid.

Another example of modification of proteins or peptides is substitution of cysteine residues preferably with alanine, serine, threonine, leucine or glutamic acid residues to minimize dimerization via disulfide linkages. In addition, amino acid side chains of peptides of the invention can be chemically modified. Another modification is cyclization of the peptide.

In order to enhance stability and/or reactivity, the protein or peptides of the invention can be modified to incorporate one or more polymorphisms in the amino acid sequence of the protein autoantigen resulting from any natural allelic

variation. Additionally, D-amino acids, non-natural amino acids, or non-amino acid analogs can be substituted or added to produce a modified protein or peptide within the scope of this invention. Furthermore, proteins or peptides of the present invention can be modified using polyethylene glycol (PEG) according to the method of A. Sehon and co-workers (Wie *et al.*, *supra*) to produce a protein or peptide conjugated with PEG. In addition, PEG can be added during chemical synthesis of a protein or peptide of the invention. Modification of proteins or peptides or portions thereof can also include reduction/alkylation (Tarr in: *Methods of Protein Microcharacterization*, J. E. Silver ed., Humana Press, Clifton NJ (1986) pp. 155-194); acylation (Tarr, *supra*); chemical coupling to an appropriate carrier (Mishell and Shiigi, eds, *Selected Methods in Cellular Immunology*, WH Freeman, San Francisco, CA (1980), U.S. Patent 4,939,239); or mild formalin treatment (Marsh (1971) *Int. Arch. Allergy and Appl. Immunol.* **41**:199-215).

To facilitate purification and potentially increase solubility of protein or peptides of the invention, it is possible to add an amino acid reporter group to the peptide backbone. For example, hexahistidine can be added via known recombinant or synthetic methods to a protein or peptide for purification by immobilized metal ion affinity chromatography (Hochuli et al. (1988) *Bio/Technol.* **6**:1321-1325). Such known methods include, e.g., cloning the nucleic acid sequences encoding the moiety to be added or synthesizing the moiety directly onto the peptide during the synthesis of the peptide. In addition, to facilitate isolation of protein or peptides free of irrelevant sequences, specific endoprotease cleavage sites can be introduced via known recombinant or synthetic methods between the sequences of the reporter group and the protein or peptide. In order to successfully desensitize an individual to a protein antigen, it may be necessary to increase the solubility of a protein or peptide by adding functional groups (e.g., charged amino acids such as Glu, Asp, or Arg) to the protein or peptide, or omit hydrophobic regions of the protein. Hydrophobic regions may be characterized by the presence of Ile, Leu, Val, Phe, and sometimes Tyr and Trp residues.

To potentially aid proper antigen processing of T-cell epitopes within a peptide, canonical protease sensitive sites can be engineered between regions, each comprising at least one T-cell epitope via known recombinant or synthetic methods. For example, charged amino acid pairs, such as KK or RR, can be introduced between regions within a peptide during recombinant construction of the peptide. The resulting peptide can be rendered sensitive to cleavage by cathepsin and/or other trypsin-like enzymes which would generate portions of the peptide containing one or more T-cell epitopes. In addition, such charged amino acid residues can result in an increase in the solubility of a peptide.

Site-directed mutagenesis of DNA encoding a peptide or protein of the invention can be used to modify the structure of the peptide or protein by methods known in the art. Such methods may, among others, include polymerase chain reaction (PCR) with oligonucleotide primers bearing one or more mutations (Ho et al. (1989) *Gene* **77**:51-59) or total synthesis of mutated genes (Hostomsky et al. (1989) *Biochem. Biophys. Res. Comm.* **161**:1056-1063). To enhance recombinant protein expression, the aforementioned methods can be applied to change the codons present in the cDNA sequence of the invention to those preferentially utilized by the host cell in which the recombinant protein is being expressed (Wada et al., *supra*).

The isolated protein and/or antigenic fragments can be used in methods of diagnosing, treating, and preventing demyelinating autoimmune responses. Thus, the present invention provides therapeutic compositions comprising isolated human MOG (SEQ ID NOS:1 and 2), or antigenic peptide fragments thereof (SEQ ID NOS:96-110 and 146-164) and a pharmaceutically acceptable carrier, excipient, or diluent. Furthermore, the isolated protein and/or fragments can be used in screening for the autoimmune disease and for developing candidates for therapeutic compositions.

Compositions Containing MOG Peptides

The invention provides compositions including at least one novel MOG peptide of the invention having SEQ ID NOS:96-110 and 146-164). Preferably, such compositions include at least two MOG peptides, at least one of which is a novel peptide of the present invention. At least one other MOG peptide having an amino acid sequence different than a novel MOG peptide of the invention may also be included. Such other MOG peptides may be any MOG peptide known in the art, for example having SEQ ID NOS:2, 42, 55, 73-80, 82, 83, 111-145, and 165-203 (see, e.g., U.S. Ser. no. 08/300,811; Bernard et al., WO 95/07096; Linington et al. (1993) Eur. J. Immunol. **23**:1364-1372; Amor et al. (1994) **153**:4349-4356; Mendel et al. (1995) Eur. J. Immunol. **25**:1951-1959; Johns et al. (1995) J. Immunol. **154**:5536-5541).

Therapeutic Pharmaceutical Compositions

One example of a composition of the invention is a therapeutic composition containing at least one MOG peptide or one MBP peptide and a pharmaceutically acceptable carrier, diluent, or excipient. In addition to compositions containing a single MOG peptide, a single MBP peptide, or MOG or MBP protein, other compositions of the invention also contain mixtures of at least two peptides (e.g., a physical mixture of at least two identical or different MOG peptides, at least two identical or different MBP peptides, or at least one MOG peptide of the invention and at least one human MBP peptide or other peptides). Therapeutic compositions may contain MOG peptides or MBP peptides containing at least one T-cell epitope of human MOG or human MBP, respectively. Still further, the therapeutic composition may contain peptides comprising at least two regions, each region comprising at least one T cell epitope of MOG and which regions may be arranged in a configuration different from a naturally-occurring configuration of the regions in human MOG. A therapeutically effective amount of one or more of such compositions can be administered simultaneously or sequentially.

Some preferred therapeutic compositions and preferred combinations of MOG peptides which can be administered simultaneously or sequentially comprise peptides comprising amino acid sequences set forth in Table 2 and having SEQ ID NOS:96-110, 146-164, and 220-223. Other preferred therapeutic compositions also include the MOG peptides shown in FIG. 2 (SEQ ID NOS:4-9, 11, 15, 16, 42-72, and 169), 5a (SEQ ID NOS. 73-80, 82,83) and 5b (the first 121 amino acids of human MOG protein SEQ ID NO. 2), more preferably, MOG 1-13 (SEQ ID NO. 42) and MOG 103-115 (SEQ ID NO. 55), as set forth in Table 1. Yet other therapeutic compositions include at least one novel MOG peptide from Table 2 as well as at least one MOG peptide having SEQ ID NOS:96-110 and 146-164 with at least one of any other known MOG peptide (having, e.g., SEQ ID NOS:42, 55, 73-80, 82, 83, 111-145, and 165-203). Yet other formulations and compositions include MBP peptides. Preferably, the MBP-containing formulations and compositions include at least one of the MBP peptides listed below in Table 3. More preferably, these MBP-containing compositions include at least one of MBP-1 to MBP-5 listed below. Most preferably, MBP-1.1, MBP-2.1, and/or MBP-4.

TABLE 3

Peptide Name	Amino Acid Sequence	SEQ ID NO:
MBP-1 (11-30)	GSKYLATASTMDHARHGFLP	
MBP-1.1 (11-29)	GSKYLATASTMDHARHGFL	
MBP-1.2 (11-31)	GSKYLATASTMDHARHGFLPR	
MBP-2 (83-105)	ENPVVHFFKNIVTPRTPPPSQGK	
MBP-2.1 (82-105)	DENPVVHFFKNIVTPRTPPPSQGK	
MBP-2.2 (82-104)	DENPVVHFFKNIVTPRTPPPSQG	
MBP-2.3 (80-98)	TQDENPVVHFFKNIVTPRT	
MBP-2.4 (82-102)	DENPVVHFFKNIVTPRTPPPS	
MBP-2.5 (80-104)	TQDENPVVHFFKNIVTPRTPPPSQG	
MBP-2.6 (80-102)	TQDENPVVHFFKNIVTPRTPPPS	
MBP-3 (111-130)	LSRFSWGAEGQRPGFGYGGR	
MBP-3.1 (111-129)	LSRFSWGAEGQRPGFGYGG	
MBP-4 (141-165)	FKGVDAQGTLSKIFKLGGRDSRSGS	
MBP-5 (101-125)	PSQGKGRGLSLSRFSWGAEGQRPGF	
MBP-A (1-20)	ASQKRPSQRHGSKYLATAST	
MBP-B (11-30)	GSKYLATASTMDHARHGFLP	
MBP-C (21-40)	MDHARHGFLPRHRDTGILDS	
MBP-D (31-50)	RHRDTGILDSIGRFFGGDRG	
MBP-E (41-60)	IGRFFGGDRGAPKRGSGKDS	
MBP-F (51-70)	APKRGSGKDSHHPARTAHYG	
MBP-G (61-80)	HHPARTAHYGSLPQKSHGRT	
MBP-H (71-90)	SLPQKSHGRTQDENPVVHFF	
MBP-I (81-100)	QDENPVVHFFKNIVTPRTPP	
MBP-J (91-110)	KNIVTPRTPPPSQGKGRGLS	
MBP-K (101-120)	PSQGKGRGLSLSRFSWGAEG	
MBP-L (111-130)	LSRFSWGAEGQRPGFGYGGR	
MBP-M (121-140)	QRPGFGYGGRASDYKSAHKG	
MBP-N (131-150)	ASDYKSAHKGFKGVDAQGTL	
MBP-O (141-160)	FKGVDAQGTLSKIFKLGGRD	
MBP-P (151-170)	SKIFKLGGRDSRSGSPMARR	
MBP-Q (82-100)	DENPVVHFFKNIVTPRTPP	
MBP-R (83-105)	ENPVVHFFKNIVTPRTPPPSQGK	
MB-S (141-165)	FKGVDAQGTLSKIFKLGGRDSRSGS	

Highly purified and isolated peptides produced as discussed above may be formulated into therapeutic compositions of the invention suitable for human therapy. If a therapeutic composition of the invention is to be administered by injection (e.g. subcutaneous injection, intravenous injection), then it is preferable that the highly purified peptide be soluble in an aqueous solution at a pharmaceutically acceptable pH (i.e. pH range of about 4-9) such that the composition is fluid and easy syringability exists. The composition also preferably includes a pharmaceutically acceptable carrier. As used herein "pharmaceutically acceptable carrier" includes any and all excipients, solvents, dispersion media, coatings, antibacterial and antifungal agents, toxicity agents, buffering agents, absorption delaying or enhancing agents, surfactants, and micelle forming agents, lipids, liposomes, and liquid complex forming agents, stabilizing agents, and the like. The use of such media and agents for pharmaceutically active substance is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the therapeutic compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

As discussed above, therapeutic compositions of the invention suitable for injectable use are preferably sterile aqueous solutions prepared by incorporating active compound (i.e., one or more highly purified and isolated peptides as described above) in the required amount in an appropriate vehicle with one or a combination of ingredients enumerated above and below, as required, followed by filtered sterilization. Preferred pharmaceutically acceptable carriers include at least one excipient such as sterile water, sodium phosphate, mannitol, sorbitol, or sodium chloride or any combination thereof. Other pharmaceutically acceptable carriers which may be suitable include solvents or dispersion medium containing, for example, water, ethanol, polyol (for example glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained for example by the use of coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. Prevention of the action of

microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosol and the like. Prolonged absorption of the injectable compositions can be brought about by including in the composition, an agent which delays absorption, for example, aluminum monostearate and gelatin.

Preferable therapeutic compositions of the invention should be sterile, stable under conditions of manufacture, storage, distribution and use and should be preserved against the contaminating action of microorganisms such as bacteria and fungi. A preferred means for manufacturing a therapeutic composition which maintains the integrity of the composition (i.e. prevent contamination, prolong storage, etc.) is to prepare the formulation of peptide and pharmaceutically acceptable carrier(s) such that the composition may be in the form of a lyophilized powder which is reconstituted in a pharmaceutically acceptable carrier, such as sterile water, just prior to use. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying, freeze-drying or spin drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

FIG. 10A and FIG. 10B show the PI for the additional MOG peptides synthesized to further define regions of high reactivity. These peptides were tested in the most recently assayed thirty-eight of the sixty-nine patients. FIG. 10A shows that peptide 41-60 elicits more T cell reactivity than peptide 41-65 and FIG. 10B shows that peptide 181-195 elicits more reactivity than peptide 171-195. Based on the previously described results, most T cell reactivity is directed toward MOG peptides 1-20, 41-60, and 181-195.

As discussed above, a therapeutic composition of the invention may comprise more than one isolated peptide. A therapeutic composition comprising a multipeptide formulation suitable for pharmaceutical administration to humans may be desirable for administration of several active peptides. The multipeptide

formulation includes at least two or more isolated peptides having a defined amino acid sequence and is capable of down regulating an antigen specific immune response. Any of the compositions described earlier which comprise at least two peptides may be suitable as a multipeptide formulation. Special considerations when preparing a multipeptide formulation include maintaining the solubility, and stability of all peptides in the formulation in an aqueous solution at a physiologically acceptable pH. This requires choosing one or more pharmaceutically acceptable solvents and excipients which are compatible with all the peptides in the multipeptide formulation. For example, suitable excipients include sterile water, sodium phosphate, mannitol or both sodium phosphate and mannitol. An additional consideration in a multipeptide formulation is the prevention of dimerization of the peptides if necessary.

Administration of the therapeutic compositions as described above to an individual, in a non-immunogenic form, can be carried out using known procedures at dosages and for periods of time effective to cause down regulation of the MOG antigen specific immune response of the individual being treated for MS. Down-regulation of an antigen specific immune response to an antigen associated with a disease condition in humans may be determined clinically whenever possible, or may be determined subjectively (i.e., the patient feels as if some or all of the symptoms related to the disease condition being treated have been alleviated).

Effective amounts of the therapeutic compositions of the invention are in the range of 1×10^5 to 2.5 mg/kg of body weight per dosage. Of course, dosages may vary according to factors such as the degree of sensitivity of the individual to the antigen, the age, sex, and weight of the individual, and the ability of peptide to cause down regulation of the antigen specific immune response in the individual. A therapeutic composition of the invention may be administered in non-immunogenic form, in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, sublingual, inhalation, transdermal application, rectal administration, or any combination of routes of

administration designed to enhance therapeutic effectiveness, or any other route of administration known in the art for administering therapeutic agents. It may be desirable to administer simultaneously or sequentially a therapeutically effective amount of one or more of the therapeutic compositions of the invention to an individual. Each of such compositions for administration simultaneously or sequentially, may comprise only one peptide or may comprise a multipeptide formulation as described above.

To administer peptide or peptide composition by other than parenteral administration, it may be necessary to coat the peptide with, or co-administer the peptide with, a material to prevent its inactivation. For example, a peptide composition may be co-administered with enzyme inhibitors or in liposomes. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP) and trasylol. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan et al. (1984) *J. Neuroimmunol.* 7:27). When a peptide is suitably protected, as described above, the peptide may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The peptide composition and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the individual's diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the composition and preparations may, of course, be varied and may conveniently be between about 5 to 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit contains between from about 10 μg to about 200 mg of active compound. The tablets, troches, pills, capsules and the like may also contain the following: a binder such as gum gragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn

starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservative, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

For injection, (subcutaneous, intravenous, intramuscular, intraperitoneal) of one or more therapeutic compositions of the invention, preferably about 1 μg - 3 mg and more preferably from about 20 μg to 1.5 mg, and even more preferably about 50 μg to 750 μg, and even more preferably about 75 μg to about 750 μg, of each active component (peptide) per dosage unit may be administered. Depending upon the regimen as described below, doses as high as 1500 μg or more may be used. It is especially advantageous to formulate parenteral compositions in unit dosage form for ease of administration and uniformity of dosage. "Unit dosage" form as used herein refers to physically discrete units suited as unitary dosages for human subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the desired pharmaceutical carrier. The specification for the novel unit dosage forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of human subjects.

Dosage regimen may be adjusted to provide the optimum prophylactic or therapeutic response. For example, several divided doses may be administered over the course of days, weeks, months or years, or the dose may be proportionally increased or reduced with each subsequent injection as indicated by the exigencies of the therapeutic situation. In one preferred therapeutic regimen, subcutaneous injections of therapeutic compositions are given once a day during an acute phase and once every other day during remission for the lifetime of the individual suffering from the disease. Alternatives would include, weekly, monthly or other periodic injections. The dosage may remain constant for each injection or may increase or decrease with each subsequent injection. A continual, lifetime treatment program may be most desirable. In the alternative, a booster injection may be administered at intervals of about three months to about one year after an initial treatment period and may involve only a single injection or may involve another series of injections similar to that of the initial treatment.

Disease Model

The effects of peptides derived from proteins of the central nervous system (CNS) have been assessed in experimental allergic encephalomyelitis (EAE), an animal model of MS. EAE is a paralytic disease which can be induced by injecting several animal species with either crude homogenates of CNS components or peptides or proteins derived from the CNS in the presence of adjuvant. In EAE, T cell autoreactivity to CNS components such as MBP is also observed. Histologically, the CNS shows signs of inflammation and demyelination. All of these features are found in MS.

EAE induced by injecting MBP into susceptible strains of rats or mice has been extensively studied: a severe, progressive paralysis of tail and limbs develops, which in mouse strains such as (PL/J x SJL)F1 can undergo remission and relapse. It has previously been shown that the injection in aqueous solution of a T cell epitope-containing peptide derived from MBP can prevent or treat EAE induced by the entire MBP molecule. It has here been discovered that the

injection of MOG, or peptides derived from MOG, as well as the injection of MBP + MOG or MBP + MOG derived peptides, can also induce EAE (Examples 3 and 4). In contrast to the effects of MBP in (PS/J x SJL)F1 mice, the injection of recombinant MOG results in a dose-dependent monophasic disease which does not relapse. Initial experiments were performed to determine which peptides constitute the T cell epitopes of MOG in SJL and (PL/J x SJL)F1 mice (described in Examples 4A and 4B below). These studies show that there appears to be two major areas of T cell reactivity to recombinant human MOG 1-121, and that each region may encompass at least one epitope. These regions are found at the N-terminal end and the C-terminal end.

Mice induced with EAE in this manner are useful as an animal model for screening potential therapies for the treatment of MS.

One method for identifying therapeutic compositions useful for the treatment of MS includes first administering human MOG to mice in immunogenic form to cause induction of EAE in the mice; then treating the mice induced with EAE with therapeutic compositions comprising at least one antigenic fragment of human MOG prior to the onset of symptoms of EAE or after the onset of symptoms of EAE in said mice; and then determining if said therapeutic composition prevents the onset or progression of the symptoms of EAE in the mice.

To demonstrate the universality of peptide therapy, experiments were set up to determine whether a T cell epitope-containing peptide derived from the MOG sequence would inhibit the induction of EAE induced by MOG in the same way that peptides derived from MBP inhibit EAE induced by MBP. These experiments are described below in Examples 4C and 4D. Furthermore, to establish a system more closely resembling MS, in which T cell reactivities to multiple CNS components are found, EAE was induced by a combination of MBP and MOG in adjuvant. The paralytic disease induced by this combination was severe and chronic, frequently resulting in death. In Example 4E, the disease induced by

MBP and MOG was treated with a MOG-derived peptide, an MBP-derived peptide or both MBP- and MOG-derived peptides.

These experiments demonstrate that various MOG peptides, alone or in combination with various MBP peptides, can reduce the clinical symptoms of EAE in mice. These results support the utility of MOG peptides and MOG peptides + MBP peptides in therapeutic compositions for the treatment of MS and other demyelinating diseases similar to EAE.

Synthetic Peptide Epitopes Complexed with MHC Class II Molecules

In the last few years the literature has described the phenomenon of the tolerization of T cell responses through the mechanism of the induction of T cell anergy, whereby specific peptide epitopes are presented to T cells by polymorphic Class II MHC molecules in the absence of cellular co-stimulation (reviewed in Mueller et al. (1989) *Ann. Rev. Immunol.* **7**:445-480). Such presentation is believed to induce T cells into a state of functional non-responsiveness. One approach taken for the induction of anergy has been to purify specific Class II glycoprotein, to allow peptide/Class II complexes to form, and to then administer these complexes to patients in order to down regulate T cell immune responses (Sharma et al., U.S. Patent No. 5,130,297). For example, one proposed treatment for autoimmune diseases involves the use of purified MHC Class II glycoproteins, or the portion of these glycoproteins capable of binding the peptide epitopes, complexed either covalently or noncovalently with specific peptide epitopes derived from a known autoantigen. However, this approach suffers from the requirement of purification of MHC Class II molecules of every haplotype known to present the peptide epitopes in question. It is now well established that most peptide epitopes are not restricted to a very small number of Class II haplotypes (reviewed in Engelhard (1994) *Ann. Rev. Immunol.* **12**:181-207), and that autoantigens can be presented by a wide spectrum of Class II molecules (Joshi et al. (1993) *Ann. Neurol.* **34**:385-393; Valli et al. (1993) *J. Clin. Invest.* **91**:616-628; Martin et al. (1991) *J. Exp. Med.* **173**:19-24. Thus, use of this approach for

the treatment of large numbers of patients would be time and resource consuming. Furthermore, this approach would be feasible only if one or a small number of disease causing peptide epitopes is identified, and if the T cell response to these few epitopes is restricted to a very small number of Class II MHC haplotypes. Such a situation does not yet and is not likely to exist in outbred human populations.

The present invention overcomes these problems through the administration of high doses of peptide(s) of the invention having T cell epitopes and allowing for the natural presentation of these epitopes by endogenous Class II molecules. Such a regimen results in anergy of T cell responses, and hence tolerization.

MOG peptides of the instant invention can be used in conjugates as disclosed, for example, in U.S. Patent 5,130,297 (Sharma et al.) where therapeutic agents are prepared using the formula X--MHC--peptide or MHC--peptide--X, wherein X represents a functional moiety selected from a toxin and a labeling group; MHC is an effective portion of the MHC glycoprotein, the glycoprotein dissociated from the cell surface on which it normally resides; and "peptide" represents any of the MOG peptides listed herein. Particularly useful MOG peptides include MOG 31-55 (SEQ ID NO:96), MOG 41-65 (SEQ ID NO:97), MOG 51-75 (SEQ ID NO:98), MOG 61-85 (SEQ ID NO:99), MOG 71-95 (SEQ ID NO:100), MOG 101-125 (SEQ ID NO:101), MOG 111-135 SEQ ID NO:102), MOG 131-155 (SEQ ID NO:103), MOG 151-175 (SEQ ID NO:105), MOG 161-185 (SEQ ID NO:106), MOG 171-195 (SEQ ID NO:107), MOG 191-215 (SEQ ID NO:108), MOG 1-25 (SEQ ID NO:109), and MOG 21-45 (SEQ ID NO:110), MOG 1-22 (SEQ ID NO:146), MOG 1-25(24C→24S) (SEQ ID NO:147), MOG 11-35 (SEQ ID NO:148), MOG 36-60 (SEQ ID NO:149), MOG 46-70 (SEQ ID NO:150), MOG 81-100 (SEQ ID NO:151), MOG 81-105 (SEQ ID NO:152), MOG 91-115 (SEQ ID NO:153), MOG 111-130 (SEQ ID NO:154), MOG 121-140 (SEQ ID NO:155), MOG 121-145 (SEQ ID NO:156), MOG 131-150 (SEQ ID NO:157), MOG 141-165 (SEQ ID NO:158), MOG 171-190 (SEQ ID NO:159), MOG 171-185 (SEQ ID NO:160), MOG 181-195 (SEQ ID NO:161), MOG 176-

190 (SEQ ID NO:162), MOG 181-200 (SEQ ID NO:163), MOG 181-205 (SEQ ID NO:164), MOG 191-210 (SEQ ID NO:104).

Other useful peptides include any known MOG peptides (see, e.g., U.S. Ser. No. 08/300,811; Bernard et al., WO 95/07096; Linington et al. (1993) Eur. J. Immuno. **23**:1364-1372; Amor et al. (1994) **153**:4349-4356; Mendel et al. (1995) Eur. J. Immunol. **25**:1951-1959; and Johns et al. (1995) J. Immunol. **154**:5536-5541).

T Cell Receptor-Derived Peptides

Several experimental animal models of autoimmune disease have associated certain T cell receptor (TCR) sequences with responses to specific autoantigen epitopes (reviewed in Gold (1994) *Curr. Opin. Immunol.* **6**:907-912). T cells bearing these particular TCRs have been shown to cause disease when transferred to naive recipients. Peptides derived from these particular TCRs have been used to immunize experimental animals, leading to the induction of an immune response directed against the specific T cells responsible for the disease. Such knowledge has led to the possibility that the control of particular TCR-bearing T cells might be accomplished through vaccination of individuals with TCR-derived peptides, which would result in the induction of regulatory T cell responses directed against the disease-causing T cells. This approach requires knowledge of specific TCR sequences recognizing disease-causing epitopes of specific autoantigens. Thus, certain peptides derived from, and capable of interacting with a TCR for human MOG or MBP are useful for treatment of MS.

Diagnosis

The proteins or peptides of the present invention can be used in "purified" form for standardization of reagents for the diagnosis and treatment of autoimmune disease. The isolated and purified protein or peptide is also useful to prepare antisera or monoclonal antibodies for use in diagnosis. An animal such as a

mouse or rabbit can be immunized with an immunogenic form of the isolated protein or isolated peptide, if necessary, conferring immunogenicity on a protein or peptide by coupling to carriers or by other techniques well known in the art. The protein or peptide can be administered in the presence of adjuvant, and progress of immunization can be monitored by detection of antibody titers in plasma or serum standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of antibodies.

Following immunization, antisera can be obtained and polyclonal antibodies isolated, if desired, from the serum. To produce monoclonal antibodies, antibody producing cells (lymphocytes) are harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Hybridoma cells can be screened immunochemically for production of antibodies reactive with the invention protein or peptide thereof. The antisera or monoclonal antibodies can be used to standardize reagents in standard assays.

Protein, peptides, or antibodies of the present invention can also be used for detecting and diagnosing autoimmune disease. For example, this could be done by combining blood, or blood products, obtained from an individual with an isolated antigenic peptide under conditions appropriate for binding of components in the blood (e.g., antibodies, HLA molecules, T-cells and B-cells) with the peptide(s) or protein, and determining the extent to which such binding occurs. Other diagnostic methods for autoimmune diseases which the protein, peptides or antibodies of the present invention can be used include paper radioimmunosorbent test (PRIST), enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA), and luminescence immunoassays (LIA).

Primers, Probes and Other Oligomers

The availability of the nucleotide sequence shown in FIG. 1 (SEQ ID NO:1) and of its complement (SEQ ID NO:2) permit the design of various oligonucleotides useful in therapeutic and diagnostic contexts. Modulation of the expression of the gene encoding MOG affects the progression of the autoimmune disease; in addition, progression can be monitored by monitoring expression using probes for RNA. Also, oligomers based on the nucleotide sequence disclosed in FIG. 1 herein can be used in standard assay methods for detecting the MOG-encoding DNA or RNA.

By oligomers "based on" the sequence disclosed in FIG. 1 (SEQ ID NO:1) is meant oligomers that contain portions of this sequence, that are complementary to the sequence or portions thereof, that represent primers used to amplify portions of the sequence when large amounts of DNA are desirable (such as for genetic manipulation) as well as oligomers designed on the basis of the disclosed sequence which effect triple helix formation with the relevant portion of the duplex representing the MOG gene. Relevant design parameters for PCR primers, oligomers capable of hybridizing to single strand targets, and oligomers capable of triple helix formation with DNA duplexes are well known in the art. Thus, oligomers "based on" the DNA of FIG. 1 (SEQ ID NO:1) may have the same sequence as a portion of this DNA, the same sequence as the complement or portion thereof, or a different sequence but one which corresponds to that disclosed in FIG. 1 (SEQ ID NO:1) through art-known design parameters.

The oligomers having nucleotide sequences based on the nucleotide sequence shown in FIG. 1 (SEQ ID NO:1) may be conventional RNA or DNA polymers, or may be modified forms thereof as generally known in the art. For example, the phosphodiester bonds of the oligomers may be substituted by alternative linkages such as phosphorothioates, methylphosphonates and the like. In addition, alternative scaffolding for nucleotide bases has also been disclosed and such modifications are included within the scope of oligomers claimed herein.

The following examples illustrate the preferred modes of making and practicing the present invention, but are not meant to limit the scope of the invention since alternative methods may be utilized to obtain similar results.

EXAMPLE 1

cDNA ENCODING HUMAN MOG PROTEIN

To obtain human DNA encoding MOG protein, a human cDNA library was subjected to the polymerase chain reaction (PCR) using internal primers designed from the published rat MOG coding sequence of Gardinier *et al.* (*supra*).

Two of the primers were homologous to the top (+) strand of the gene (primers 94-111 and 166-183 (SEQ ID NO:34), base 1 starting at the ATG) and two were homologous to the bottom (-) strand of the gene (primers 538-555 (SEQ ID NO:35) and 685-702). The combination of primers 166-183 (SEQ ID NO:34) and 538-555 (SEQ ID NO:35) was successful in effecting the amplification of a fragment of the approximately 400 bp expected size from a human brain cDNA library. The sequence of these primers was:

166-183: CAGAATCC<u>GGGAAGAATGCCACGGGC</u> (SEQ ID NO:34);

and

538-555: CAGCGGCCGC<u>ACGGAGTTTTCCTCTCAG</u> (SEQ ID NO:35).

An EcoRI site is present in the 166-183 primer (SEQ ID NO:34); and a NotI site is present in the 538-555 primer (SEQ ID NO:35). The 400 bp PCR product was cloned into expression vector pVL1393 by digesting pVL1393 (Pharmingen CA) with EcoRI and NotI, digesting the amplified product with the same enzymes and ligating the resulting fragments. The insert was verified by digesting several clones derived from the ligated plasmids with EcoRI and NotI and sequencing the resulting 400 bp human MOG fragment. The resulting insert

putatively lacks 184 bp of 5' sequence and 201 bp of 3' sequence, based on the 738 bp rat open reading frame. Two primers were designed from the 400 bp insert from positions 346-363 top and bottom strands as follows:

5'-CAGAATTCTCAGGTTCTCAGATGAAGGA-3' (SEQ ID NO:36); and

5'-AAGCGGCCGCTATCCTTCATCTGAGAACCT-3' (SEQ ID NO:37).

wherein an EcoRI site is present in the first strand and a NotI site in the second. Underlined regions correspond to the MOG sequence.

The human MOG 346-363 top and bottom primers (SEQ ID NOS:36 and 37) were used in combination with the above-mentioned 3' rat primer to amplify the 3' missing end of the gene from the same human brain cDNA library as previously used. A PCR product corresponding the 3' end of the gene was obtained. This 3' fragment obtained had the expected 400 bp size and this fragment was cloned in pVL1393 and sequenced.

To obtain the 5' portion of the gene, a human brain medulla λgt10 library obtained from Clontech which had been previously amplified and had a titer of $8x10^{10}$ pfu/ml was screened following the protocol described by the manufacturer. The library was plated onto 12 large plates at 30,000 plaques/plate and the plaques were lifted onto nitrocellulose filters (2 replica filters/plate). Twelve filters lifted from the 12 different plates were then hybridized to a ^{32}P labelled probe corresponding to the human MOG internal 400 bp fragment initially cloned (positions 184-534). Twenty-two strong positives were obtained. A plug was picked for each positive from the original plates and incubated overnight with λ dilution buffer to elute the phage from the agar. The tube was then centrifuged and the supernatant transferred.

The DNA was amplified from each individual pool using either a λgt10 forward primer with an SstII site:

5'-CTTTTGAGCAAGTTCAGCCTGGTTAAG-3' (SEQ ID NO:38)

or a λgt10 reverse primer with an XhoI site:

5'-ACCTCGAGGAGGTGGCTTATGAGTATTTCTTCCAGGGTA-3' (SEQ ID NO:39)

as well as a human MOG internal primer top or bottom strand:

5'-GGTGCGGGAAAGGTGACTCTCAGGATCCGGAAT-3' (SEQ ID NO:40) or

5'-ATTCCGGATCCTGAGAGTCACCTTTCCCGCACC-3' (SEQ ID NO:41).

The last two primers (SEQ ID NOS:40 and 41) include a BamHI site (underlined in the sequences) naturally present in the human MOG sequence.

The primers were used in four different combinations: 1) forward top/internal MOG bottom; 2) reverse bottom/internal MOG bottom; 3) internal MOG top/reverse bottom; and 4) internal MOG top/forward top.

The first two combinations provided the 5' end of the gene (up to the BamHI site) and the last two, the 3' end of the gene. Both 5' and 3' portions include untranslated regions. Which of the two members of each combination actually resulted in the desired fragment depends on the orientation of the cDNAs cloned into λgt10.

The size of the fragments obtained varied from one pool to another. Five of the largest 5' fragments or 3' fragments were subcloned into the SstII and BamHI or BamHI and XhoI sites of the SK polylinker. Three clones from each pool were then sequenced to rule out the presence of PCR errors. This provided

the complete sequence of the gene coding region as well as 174 bp of the 5' untranslated sequence.

The complete DNA sequence recovered (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) are shown in FIG. 1. The human MOG gene encodes a preprotein of 248 amino acids which has 87% homology with the 246 amino acids in the rat protein. The mature protein contains 218 amino acids, numbered 1-218 in FIG. 1 (SEQ ID NO:2). The mature protein begins at the glycine shown at position 1 and is derived from the 248 amino acid preprotein by cleavage from the presequence extending from the MET start codon to the alanine residue immediately preceding the glycine shown in position 1.

Human MOG cDNA was also cloned into a pET H6 vector Novagen, Madison, WI for expression in E. coli. pET.H6 contains a sequence encoding six histidines which allows for purification of any recombinant protein over a Ni2+ column. A truncated human MOG cDNA (no leader sequence and no transmembrane domains) encoding amino acids 1-121 of human MOG (the first 121 amino acids of SEQ ID NO. 2) was amplified by PCR using the following oligonucleotides:

5' primer:
5'-AGCTCGAGCCGCGGAGGGCAGTTCAGAGTGATA-3' (SEQ ID NO:94)

3' primer:
5'-GACTCGAGTCACCAGTAGAAAGGATCTTC-3' (SEQ ID NO:95)

The 363 bp PCR fragment was then cloned between the unique SstII and XhoI sites of pET.H6. After cloning, the cDNA was entirely sequenced.

EXAMPLE 2
EXPRESSION OF TRUNCATED HUMAN MOG

A. Expression in Sf-9

The PVL1393 transfer vector containing the truncated human MOG cDNA encoding amino acids 1-121 of human MOG (the first 121 amino acids of SEQ ID NO. 2) was cotransfected into Sf-9 cells along with Baculogold linearized Baculovirus DNA (Pharmingen, San Diego, CA). The culture supernatant containing recombinant viruses was harvested after 4 days. The recombinant virus was plaque purified and subjected to 3 rounds of amplification to obtain a high titer viral stock. Sf-9 cells were then infected with the viral stock at a MOI of 2.0. The supernatant from infected cells was harvested 48 hours after infection and applied to a NiNTA agarose column. The recombinant MOG protein was eluted under non-denaturing conditions using 250 mM Imidazole, dialyzed against 5% propionic acid and H_2O and subsequently lyophilized. The protein concentration was estimated by BCA. The purified MOG protein was visualized on a 12.5% polyacrylamide gel stained with Coomassie blue.

B. Expression in *E. coli*

The pET.H6 vector containing the truncated human MOG cDNA was introduced into BL21(DE3) cells (Novagen, Madison, WI.) by transformation. Several colonies were grown together in 2YT medium to an OD of 1.0. The bacteria were then induced overnight with 1 mM IPTG. Cells were harvested and lysed with 6 M Guanidine/100 mM Tris-HCl, pH 8.0 at room temperature overnight. The lysate was centrifuged at 20.000 rpm for 30 minutes and the resulting supernatant applied to a NiNTA agarose column (Quiagen, Chatsworth, CA). The protein was eluted with 6 M Guanidine/100 mM sodium phosphate, pH 4.5, dialyzed first against 5% propionic acid, then against H_2O and subsequently lyophilized. The protein concentration was estimated by BCA. The purified

MOG protein was visualized on a 12.5% polyacrylamide gel stained with Coomassie blue.

EXAMPLE 3
INDUCTION OF EAE

Two groups of (PL/J x SJL)F1 mice were injected with 10 μg and 50 μg respectively N-terminal fragment of recombinant human MOG expressed in insect (-TM, recombinant, Sf-9) which was prepared according to Example 2A herein. The truncated MOG contains amino acids 1-121 (hereinafter intended to refer to MOG 1-121) and was selected, in part, because of its solubility relative to the solubility of amino acids 122-218 of the MOG protein (SEQ ID NO:2). Human MOG 122-218 (residues 121-218 of SEQ ID NO. 2) is extremely hydrophobic and is believe to include two transmembrane regions. MOG 1-121 was emulsified in complete Freund's adjuvant and injected subcutaneously in mice. Emulsions were prepared using 1 volume of PBS or water containing the stated quantity of MOG 1-121, as described above combined with one volume of CFA (Life Technologies, Grand Island, NY) containing 400 μg H37Ra (Difco Laboratories, Detroit, MI) for a total infection volume of 100 ul per mouse. At the same time as the MOG 1-121 injection, 200 ng pertussis toxin (JRH Biosciences, Lenexa, KS or List Biomedical Labs., Campbell, CA) was also injected intravenously. The 200 ng pertussis toxin i.v. injection was repeated 2 days later. Beginning at Day 8 after initial immunization, the mice were observed for signs of paralysis and scored daily as an indicator that EAE has been induced. Scoring was based on clinical signs according to the following scale: 1, tail paralysis; 2, partial hind limb paralysis; 3, complete hind limb paralysis; 4, forelimb paralysis; 5, moribund or dead.

The onset of symptoms began as early as 14 days for some mice. The mice were observed for 31 days, at which time the mice were sacrificed, the brains and spinal cords were harvested, and histological studies were undertaken to

verify the clinical observations. Sixty percent (60%) of mice immunized with 50 μg of MOG (-TM, recombinant, Sf-9) and eighty percent (80%) of mice immunized with 10 μg of recombinant MOG (-TM, recombinant, Sf-9) exhibited symptoms of EAE.

This procedure was repeated substituting recombinant N-terminal fragment of recombinant human MOG 1-121 expressed in *E. coli* (-TM, recombinant, *E. coli*) for recombinant N-terminal fragment of recombinant human MOG 1-121 expressed in insect cells (-TM, recombinant, Sf-9). Preliminary results indicate similar findings.

EXAMPLE 4
T CELL RESPONSES AND PEPTIDE TREATMENT OF MURINE EAE MODEL

A. (PL/J x SJL)F1 Mice with MOG-Induced EAE

(PL/J x SJL)F1 mice (obtained from Jackson Lab, Bar Harbor, ME) were immunized at the base of the tail with 100 μl of a 1:1 emulsion of 400 μg recombinant MOG in PBS with Complete Freund's Adjuvant (CFA). Eight to ten days later, mice were sacrificed and a single cell suspension of lymph node cells was prepared. Triplicate wells containing 4 x 10^5 cells were incubated with 0-100 μg/ml of a set of MOG-derived 20mers spanning the length of recombinant MOG (peptides 1-20 (SEQ ID NO:73), 11-30 (SEQ ID NO:74), 21-40 (SEQ ID NO:75), 31-50 (SEQ ID NO:76), 41-60 (SEQ ID NO:77), 51-70 (SEQ ID NO:78), 61-80 (SEQ ID NO:79), 71-90 (SEQ ID NO:80), 81-100 (SEQ ID NO:151), 91-110 (SEQ ID NO:82), 101-120 (SEQ ID NO:83), and 111-130 (SEQ ID NO:154)). Triplicate wells were also cultured with 2.5 μg/ml Concanavalin A (Sigma, St. Louis, MO) which stimulates all T cells, 100 μg/ml purified protein derivative (PPD), the antigen expressed by mycobacteria in the CFA or 1-100 μg/ml of the immunizing antigen, recombinant MOG. These three sets served as positive controls for T cell responses.

Cells were cultured in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (Sigma, St. Louis, MO) or 0.5% fresh normal mouse serum, supplemented with 10 mM glutamine, 100 U/ml penicillin, 100 U/ml streptomycin and 2 x 10^{-5} M 2-mercaptoethanol. Cultures were maintained at 37°C and 5% CO_2, for various periods of time. To assess proliferative responses, cultures were maintained for 96 hr with 1 μCi ^{3}H-thymidine (ICN Pharmaceuticals, Inc., Irvine, CA) added for the final 12-16 hours. Cells were then washed onto glass-fiber mats and ^{3}H-thymidine incorporation into DNA measured by scintillation counting. To assess production of the cytokine Il-2, cultures were maintained for 24 hours, after which 50 μl of culture supernatant was transferred to new wells containing 5 x 10^3 CTLL2.3 cells, an IL-2 dependent, IL-4 independent cell line (Dr. David Raulet, UCal Berkeley). The CTLL2.3 cultures were maintained for 48 hours. One μCi of ^{3}H-thymidine was added for the final 24 hr of the culture period and incorporation into DNA was assessed as described above for proliferative responses. Standard curves for known levels of recombinant IL-2 were run in parallel.

Peptides 1-20 (SEQ ID NO:73), 11-30 (SEQ ID NO;74), 31-50 (SEQ ID NO:76, and 41-60 (SEQ ID NO:77) stimulated proliferative responses at least twice the background (i.e., had a Stimulation Index [SI] > 2) at two or more of the tested peptide concentrations. Responses to the positive controls recombinant MOG and PPD were very strong. A similar pattern of peptide effectiveness was observed for IL-2 secretion. Peptides 1-20, 11-30, 31-50 and 41-60 stimulated T cell responses with SI > 2 at at least two concentrations of tested peptide.

B. SJL Mice with MOG-Induced EAE

The experiment described in (A) above, was repeated in the SJL strain of mouse (obtained from Jackson Lab, Bar Harbor, ME). Lymph node cells from MOG-primed SJL mice were incubated with 0-50 μM concentrations of the set of MOG-derived 20mers described above. Proliferation was assessed as described above.

The highest responses over the background (no antigen) were found in wells stimulated with peptides 1-20 (SEQ ID NO:73), 91-110 (SEQ ID NO:82), and 81-100 (SEQ ID NO:151); each of these peptides stimulated responses that were five-fold greater than the background response (i.e., had an SI > 5) with at least one concentration of peptide. Peptide 11-30 (SEQ ID NO:74) stimulated a T cell response with an SI of approximately 2. Thus, there appear to be two major areas of T cell reactivity to recombinant human MOG in SJL mice, and each region may encompass at least one epitope. These regions are found at the N-terminus of the molecule at amino acids 1-30 and at the C-terminal end at amino acids 81-110.

C. SJL Mice with MOG-Induced EAE

Two groups of SJL mice were immunized at the base of the tail with 200 μl of a 1:1 emulsion of 200 μg recombinant MOG in PBS with CFA, supplemented with 400 μg H37Ra *Mycobacterium tuberculosis* (Difco, Detroit, MI). 200-400 ng Pertussis Toxin was also injected i.v. on day 0 and day 2. One group of mice was injected i.v. with 250 nmoles MOG 91-110 (SEQ ID NO:82) in aqueous solution and the other group was injected with PBS on days 7, 9, 12 and 15 following immunization. Mice were scored daily to assess their degree of paralysis, using the scale described above.

Mice injected with PBS developed severe EAE around day 10 which remained at a high level for 30 days (mean clinical score of 3-4). In contrast, mice injected with MOG 91-110 did not develop as severe a disease (mean clinical score approximately 1) and the disease remitted to a mean clinical score of almost zero. In addition, MOG 91-110 decreased disease incidence (60% vs. 100%) and decreased the mortality which accompanied the severe disease (27% vs. 50%). By these criteria, the peptide MOG 91-110 reduced the severity of EAE induced by recombinant MOG in SJL mice.

D. (PL/J x SJL)F1 Mice with MOG-Induced EAE

EAE was induced in (PL/J x SJL)F1 mice by injecting 100 μl of an emulsion containing 400 μg recombinant MOG in CFA supplemented with *Mycobacteria* and pertussis toxin, as described above. At days 6, 8, 10, 12, 14, and 16 following immunization, groups of 10 mice were injected with 250 nmoles of the peptide MOG 41-60 (SEQ ID NO:77) in aqueous solution or with PBS. Mice injected with MOG 41-60 had delayed disease onset, reduced mortality, and lower average daily clinical scores after treatment compared to PBS-injected mice. Thus, in (PL/J x SJL)F1 mice, the peptide MOG 41-60 was effective at treating EAE induced by MOG.

E. (PL/J x SJL)F1 Mice With MBP+MOG-Induced EAE

(PL/J x SJL)F1 mice were immunized with a mixture of 100 μg recombinant MOG and 75 μg guinea pig MBP emulsified together in CFA, as described above. MBP was prepared from guinea pig spinal cords by a modification of the method of Smith (*J. Neurochem.* (1969) **16**:83). Briefly, MBP was extracted from isolated myelin membranes using chloroform and methanol, precipitated with potassium citrate, acid extracted and lyophilized. SDS-PAGE analysis of this material showed a major band at 18.5 kD.

Mice injected with MBP and MOG were scored daily based on clinical signs according to the scale described above. Mice were injected i.v. with 125-500 nmoles of MBP Ac1-11[4Y] (Sequence Ac-ASQYRPSQRSK (SEQ ID NO:204)), 125-500 nmoles MOG 41-60 (SEQ ID NO:77), or a combination of 125 or 250 nmoles of each peptide in aqueous solution. Control mice received PBS. All mice were injected 7 times every other day starting around day 7 following immunization.

Disease induced by injecting both recombinant human MOG and MBP developed around day 10, was severe and chronic and resulted in high mortality.

Mice treated with combinations of 125 and 250 nmoles each MOG 41-60 and MBP Ac1-11 [4Y] reduced disease severity by several criteria: later day of disease onset, reduced clinical scores both during treatment and after the cessation of therapy, and decreased mortality. MBP Ac1-11[4Y] alone also reduced disease severity by the same criteria. MOG 41-60 at 250 nmoles reduced disease severity as assessed by reduced clinical scores during the treatment regimen. Thus, a peptide derived from MOG and a peptide derived from MBP, as well as a combination of both peptides, can treat disease induced by both MBP and MOG.

EXAMPLE 5

T cell RESPONSES TO HUMAN MOG PEPTIDE

Peptides were synthesized using either an Applied Biosystems peptide synthesizer (Foster City, CA) or an Advanced Chemtech robotics system (Louisville, KY) utilizing FastMOC™ chemistry with commercially available Wang resins (Advanced Chemtech, Louisville, KY or Bachem, Basel Switzerland), and Fmoc protected amino acids as described previously by Hill et al. (*J. Immunol.* (1994) **52**:2890-2898).

T cell assays were performed to further refine the identification of T cell epitopes. Peripheral blood lymphocytes were isolated from the blood of a human volunteer HLA-DR2 positive donor using Ficoll Hypaque. Twenty million lymphocytes were seeded into 96 wells of a microtiter dish at 2×10^5 per well in RPMI culture media supplemented with human AB serum. A mixture of selected MOG peptides listed in Table 1 was added at a final concentration of 50 μM for each peptide. The MOG peptides selected were both naturally occurring peptides and analogs of naturally occurring peptides.

Cultures were incubated in a humidified CO_2 at 37°C for twelve days, and were intermittently supplemented with human IL-2 (20 U/ml) and IL-4 (5 U/ml) (Collaborative Biomedical Products, Bedford, MA). A sample from each culture well was removed, washed to remove previously added peptide, and reseeded into

four wells of a fresh microtiter dish (two wells with the peptide mixture and two wells without the peptide mixture for each sample). Autologous irradiated cryopreserved lymphocytes were added as antigen presenting cells. After further 3 days of incubation, ^{3}H-thymidine incorporation was measured. Positive microtiter lines were scored if the mean incorporation in the peptide wells was greater than or equal to 1.5-fold higher than the wells without peptide. Positive microtiter lines were expanded with IL-2 and IL-4, and then reassayed the following week by the same methods with the individual peptides rather than the peptide mix.

Using this method, MOG 1-13 (SEQ ID NO:42) 13mer and MOG 103-115 (SEQ ID NO:55) 13mer showed the strongest response. FIG. 6 shows the results of the assays performed.

EXAMPLE 6

HUMAN MS PATIENT T cell RESPONSE TO MOG PEPTIDES AND N-TERMINAL FRAGMENT OF RECOMBINANT HUMAN MOG

A. Responses to 20mer Peptides

Peptides (20 amino acids in length) were synthesized using either an Applied Biosystems peptide synthesizer or Advanced Chemtech robotics system utilizing FastMOC™ chemistry with commercially available Wang resins, and Fmoc-protected amino acids as described previously by Hill et al. (*J. Immunol.* (1994) **152**:2890-2898). These peptides are 20 amino acid peptides of human MOG overlapping by 10 amino acids and are shown in FIG. 5a.

Following the procedures described above, the T cell response of a human patient suffering from MS was tested with the group of MOG 20mer peptides shown in FIG. 5a and a N-terminal fragment of recombinant human MOG 1-121 expressed in insect Sf-9 (-TM, recombinant, Sf-9), which is shown to induce EAE

in mice in Example 3. One or more of the MOG peptides tested are suspected of containing at least one T cell epitope for the autoantigen responsible for MS.

The PBLs of the patient were cultured in microtiter wells according to the protocol above, except that both recombinant MOG 1-121 (48 of 96 wells) and a mixture of 10 MOG fragments (20mer peptides shown in FIG. 5a) (48 of 96 wells) were used separately to initiate the cultures. A sample from each culture well was removed, washed to remove previously added peptide, and reseeded into six wells of a fresh microtiter dish. Six microtiter wells were subsequently found to react with both recombinant MOG and the peptide mixture (2 wells from the cultures initiated with recombinant MOG and 4 wells from the cultures initiated with the peptide mixture). The results confirm that MS patients can have T cells which can be activated by MOG and which recognize one or more T cell epitopes contained in the MOG peptide mixture. There were also a number of wells that reacted with the peptide mixture but not the recombinant MOG. The significance of these positives is less clear but it is believed that true MOG reactive T cells which recognize an epitope in MOG which is not readily processed *in vitro*.

B. Responses to 25mer Peptides

The T cell responses of twenty-one MS patients were tested with eight MOG 25mer peptides derived from the N-terminal portion of MOG. The peripheral blood lymphocytes (PBL) of MS patients were prepared and cultured according to the protocol above, except that cultures were initiated with 50 μg/ml of the N-terminal fragment of recombinant human MOG 1-121 (SEQ ID NO:2), prepared according to the protocol in Example 2. After the expansion of the microtiter cultures with IL-2 and IL-4, the entire contents of each well was harvested, washed to remove residual MOG, and reseeded into twelve wells of a fresh microtiter dish. Each culture was tested (in duplicate wells) with no antigen, 50 μg/ml recombinant human MOG 1-121 (the initiating antigen), or 25 μM each of four of the N-terminal MOG 25mer peptides listed in Table 2. Peptide Test Group I included 1-25 (SEQ ID NO:109), 11-35 (SEQ ID NO:148), 21-45 (SEQ

ID NO:110), and 31-55 (SEQ ID NO:96). Peptide Test Group II included 41-65 (SEQ ID NO:97), 51-75 (SEQ ID NO:98), 61-85 (SEQ ID NO:99), and 71-95 (SEQ ID NO:100). Autologous irradiated cryopreserved lymphocytes were added as antigen presenting cells. After a further three days of incubation, ^{3}H-thymidine incorporation was measured. Positive microtiter lines were scored if the mean incorporation in the peptide wells was greater than or equal to 3-fold higher than the wells without peptide, and the incorporation in the peptide wells was at least 500 counts per minute higher than that in the wells without peptide.

Nineteen of the 21 patients produced cultures responding to recombinant human MOG 1-121, and these 19 produced a total of 133 MOG-responding T cell lines. Ninety-two lines were tested on the Group I peptides and 42 were tested on the Group II peptides outlined above. Peptides 1-25, 41-65, and 71-95 all elicited responses from more than 7% of the lines tested on them, and therefore would be considered major T cell epitopes. All of these represent significant levels of reactivity.

EXAMPLE 7
PURIFICATION OF NATIVE MOG

A. Myelin Membrane Preparation

The procedures of myelin preparation were adapted from Smith (*J. Neurochem.* (1969) **16**:83-92). Human brain white matter was homogenised in a blender at the highest speed setting (about 1:2 wt/v) in 10.5% sucrose (wt/v) containing 3 mM EDTA, 20 μg/ml leupeptin, 10 μg/ml soybean trysin inhibitor (STI) and 1 mM phenyl-methylsulfonyl fluoride (PMSF) (solution A). The homogenate was centrifuged at about 17,000 x g for 45 min. at 4°C. The red blood cell and yellow histone portion of the resulting pellet was carefully removed with a spatula and the remaining pellet was homogenised in the blender at lowest speed in about 2x original volume with cold 30% sucrose containing EDTA and protease inhibitors as above. The homogenate was poured into polycarbonate

ultracentrifuge tubes and an overlay of 10.5% sucrose was added. The myelin membranes were collected at the interface after centrifugation (68,000 x g for 50 min. at 4°C). The myelin was washed once with deionized water (DDW) and pelleted at 68,000 x g for 60 min. at 4°C. The resulting pellet was resuspended in a small volume of DDW and stored in aliquots at -80°C for further purification.

About 1 g of myelin membranes, as determined by the Peterson Protein Assay (Peterson (1977) *Anal. Biochem.* **83**:346-356) was thawed out and brought to a final volume of 100 ml with DDW with protease inhibitors (solution A). The membranes were delipidated with 500 ml $CHCl_3$:MeOH (2:1 v/v) by shaking in a 1-liter flask. After centrifugation at 14,000 x g for 20 min., the proteins at the interphase layer were collected and dried in the hood with a fan on until all the organic solvent had evaporated. The pellet was resuspended into 50 ml $CHCl_3$:MeOH (2:1 v/v) by homogenization with a motor driven Potter-Elvehjem homogenizer. After centrifugation in Teflon tubes (14,000 x g for 20 min.), the solvent was decanted and the pellet was dried in the hood as above.

B. Solubilization

Each of the pellets in the Teflon tubes was resuspended in 25 ml LDAO buffer (50 mM Hepes, pH 7.6, 0.5 mM DTT, 90 mM NaCl, 1.2 % lauryldimethyl amine oxide, 5 mM EDTA, 20 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor and 1 mM phenyl-methylsulfonyl fluoride) and first tip sonicated for 3 x 10 sec. each at 4°C. Then the whole mixture was brought to 0.5 liter with LDAO buffer and sonicated in a bath sonicator for 15-30 min. The solubilized material was separated by ultracentrifugation (100,000 x g for 30 min.). The pellet was resolubilized in 50 ml LDAO buffer sonicated (tip and bath), and centrifugation was repeated as above. Both supernatants were combined and stored at -80°C for further purification.

C. Affinity Chromatography

Anti-MOG specific monoclonal antibody, 818C5 (Abo et al. (1993) *Biochem. Mol. Biol. Int.* **30**:945-958) was used as the major chromatographic step in the purification of MOG. Purified mAb 8-18C5 was dialyzed against Sulfolink Sample Preparation Buffer (0.1 M sodium phosphate, 5 mM EDTA, pH 6.0 at 4°C). The antibodies were reduced with 2-mercaptoethanolamine and desalted with SWIFT Desalting columns (Pierce, Rockford, IL) according to the manufacturer's specifications. The IgG was coupled to the Sulfolink Gel (Pierce, Rockford, IL) at 5 mg IgG/ml resin. The slurry was mixed for 15 min. at room temperature and then incubated, without mixing, for at least 30 min., also at room temperature. After washing the gel twice with 50 mM Tris, 5 mM EDTA, pH 8.5, the non-specific binding sites on the gel were blocked by cysteine-HC1, followed by low and high pH washes according to the manufacturer's procedures (Pierce). The resin was then equlibrated with 0.2 M sodium borate, pH 9.0 and 18 M dimethylpimelimidate was added to the mix, which was rocked for 30 min. at room temperature to crosslink the antibodies. The reaction was stopped by incubating the gel in 0.2 M ethanolamine, pH 8.0 for one hour.

Two 25 ml 8-18C5 Sulfolink columns were packed and all the procedures were performed at 4°C. The new columns were "aged" by going through 2 cycles of Chromatography Buffers (Buffer #1: 50 mM HEPES, 0.5 mM DTT, 1 mM PMSF, 20 μg/ml leupeptin, 10 μg/ml STI, 90 mM NaCl, pH 7.6); Buffer #2: (same as #1, plus 3 mM EDTA & 0.4% LDAO); Buffer #3: (50 mM HEPES, 0.5 mM DTT, 1 mM PMSF, 20 μg/ml leupeptin, 10 μg/ml STI, 0.5 mM EDTA, 0.5 M NaCl, 0.04% dodecyl maltoside, pH 7.6); Buffer #4: (same as #3 but no STI); Buffer #5: (150 mM glycine, 1 mM PMSF, 5 ug/mL leupeptin, 0.04 % dodecyl maltoside, pH 2.0); Buffer #6: 50 mM HEPES, pH 7.6).

About 1-1.5 liter of extract was passed over a 25 ml column. The LDAO extract was diluted two fold with dilution buffer (i.e. the LDAO buffer without LDAO) to decrease the concentration of LDAO to 0.6% and tip sonicated for 3 -

5 sec. After sonication, the material was loaded onto the 8-18C5 Sulfolink columns, which had been equilibrated with Buffer 1, followed by Buffer 2. After loading, the columns were washed with Buffer 3, followed by Buffer 4. MOG was eluted with Buffer 5. Fractions (4 ml) were collected in tubes containing 20 μl of 3 M Tris, pH 9.0 to bring the pH to about neutral. The fractions were pooled according to SDS-PAGE analysis. Purity of the protein was determined by N-terminal sequencing.

Three to four runs of affinity purified material with purity $\geq$ 90 %, as determined by N-terminal sequencing, were pooled and further loaded onto a DEAE-Sepharose FF column (Biotech, Inc., Piscataway, NJ), which was pre-equilibrated in 50 mM HEPES, 1 mM DTT, 1 mM PMSF, 5 μg/ml leupeptin, 50 mM NaCl, 0.04 % dodecyl maltoside, pH 7.6 The flow-through material together with 1 column wash were collected and dialyzed extensively against DDW with Spectra #2 dialysis tubing (VWR, Boston, MA) with a molecular weight cutoff of 12-14 kD. Most of the MOG protein was recovered in the precipitate. The protein in the supernatant was concentrated by lyophilization. Both the precipitate and the lyophilized material were combined together, washed a couple of times with DDW and stored as a suspension in DDW. Total proteins were determined by amino acid analysis and purity was determined by N-terminal sequencing.

EXAMPLE 8
T CELL EPITOPE MAPPING OF HUMAN MOG

In order to establish MOG-reactive T cell lines for epitope mapping, peripheral blood mononuclear cells were isolated from the blood of 46 MS patients recruited at the Rocky Mountain MS Center (Denver, CO) and Fairview Medical Center (Minneapolis, MN). These patients were selected based on the following criteria. Newly diagnosed, relapsing-remitting, and chronic-progressive MS patients were included in the study. MS patients were included only if they had disease activity within the previous two years. Patients were excluded if they had

received steroids or other immunosuppressive medications within the four months prior to the study.

MOG-reactive T cell lines were initiated from each patient by culturing 10 x 10^6 mononuclear cells in 2.5 ml of complete medium. On average, three cultures were set up for each subject, which were pooled before analysis. To ensure detection of naturally processed epitopes, the cultures were stimulated with intact MOG (6 μg/ml, >90% pure) isolated from human brain according to the protocol described in Example 7, and were supplemented after six days of culture with IL-2 (Collaborative Biomedical Products, Becton Dickinson Labware, Bedford, MA) and IL-4 (Collaborative Biomedical Products, Becton Dickinson Labware, Bedford, MA). After two additions of IL-2 and IL-4, the individual T cell lines were assayed on day 14-18 for proliferative responses to titrating doses of MOG, an overlapping set of MOG peptides, or medium alone as a control. Twenty thousand T cells from each T cell line were challenged in triplicate in a volume of 200 μl with titrations of each MOG peptide or MOG protein in the presence of 5 x 10^4 gamma-irradiated (3500 Rads) peripheral blood mononuclear cells as antigen presenting cells (APC). After 3 days of culture at 37°C, each microwell received 1 μCurie titriated thymidine (ICN Radiochem Irvine, CA) for an additional 16-24 hours. Thymidine incorporation was then measured by liquid scintillation counting. The response to MOG or each MOG peptide is expressed as stimulation index (SI), which is the ratio of the tritium counts per minute (CPM) incorporated in the presence of antigen to the tritium CPM incorporated in medium alone as a negative control.

Overlapping peptides spanning the length of the MOG sequence were synthesized as described in Example 5, purified, and resuspended at 1 mM in PBS pH 7.2. One representative set of peptides used for epitope mapping is shown in FIG. 7. Eighteen peptides, 20 or 25 amino acids in length with overlaps of 5, 10 or 15 amino acids were tested for the presence of T cell epitopes by the challenge of MOG-reactive T cell lines described above.

Peripheral blood T cell reactivity to MOG, and to peptides spanning the MOG sequence, was evaluated in 46 MS patients. Thirty-one of these 46 cultures (67%) responded to MOG protein with stimulation indices of 2 or greater in secondary proliferation assays, and were used to assess reactivity to MOG peptides. Reactivity of the T cell lines to MOG peptides was evaluated based on the percent of MS patients recognizing each peptide and on the relative magnitude of the responses to each peptide. In this preliminary map, three distinct regions of high reactivity were identified across the MOG molecule. Responses of these T cell lines to the set of MOG peptides were assessed by sum of ranks analysis, in which the highest three responses of each cell line to peptides are ranked from highest to lowest (3,2,1). The ranks received by each peptide were then summed across the population, representing the relative strength and frequency of the responses to each individual peptide. As shown in FIG. 8A, major T cell epitopes are located in the N-terminal third of the MOG molecule, with other major areas of T cell reactivity at amino acids 111-135 and 171-195. FIG. 8B shows the mean percentage of T cell reactivity accounted for by each of the peptides in this population of 31 MS patients.

By this method of analysis, the same regions (1-95, 111-135, and 171-195) show the highest reactivity. These results suggest that combinations of more than one MOG peptide of the invention have the potential to down-regulate the immune response in subjects afflicted with MS.

EXAMPLE 9

ADDITIONAL T CELL EPITOPE MAPPING OF HUMAN MOG

In order to re-establish and further define major areas of T cell activity to the human MOG molecule, peripheral blood mononuclear cells were isolated from an additional 49 MS patients recruited at the same medical centers and selected by the same criteria as mentioned in Example 8. These additional T cell lines were established and assayed according to Example 8.

Additional peptides 20-25 amino acids in length shown below in Table 4 were tested. These peptides completed an overlapping set of 25 amino acid long peptides which span the entire MOG molecule, overlapping by 15 amino acids. Also, a few peptides 15-22 amino acid long were included to further define the major areas of T cell reactivity identified in Example 8.

TABLE 4

Amino Acids (peptides)	SEQ ID NO:
1-25	109
1-22	146
21-45	110
41-60	77
36-60	149
46-70	150
81-105	152
91-115	153
121-145	156
141-165	158
171-185	160
176-190	162
181-195	161
181-205	164

Peripheral blood T cell reactivity to MOG, and to peptides spanning the MOG sequence, was evaluated in a total of 95 MS patients, 46 patients from Example 8. Sixty-nine of the 95 cultures (73%) responded to MOG protein in secondary proliferation assays, and were used to assess reactivity to MOG peptides as described in Example 8. The criteria used to define a positive response included an SI of 2 or greater, difference between antigen wells and medium wells to be 500 CPM or greater and standard deviation of antigen wells less than or equal to the difference in CPM between antigen wells and medium wells.

Reactivity of the T cell lines to MOG peptides was evalulated by a method referred to as positivity index (PI). PI for a peptide is the mean SI multiplied by the percentage of positive responses to that peptide. PI takes into account both the SI (strength of response) and the frequency of response for each peptide. FIG. 9 shows the PI for each of the peptides in the total population of sixty-nine MS patients. By this method of analysis, the regions of highest reactivity are 1-65 and 171-195, with other major areas of T cell reactivity at amino acids 61-85, 101-135, and 191-215.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

We claim:

1. A peptide of human MOG having an amino acid sequence selected from the group consisting of MOG 31-55 (SEQ ID NO:96), MOG 41-65 (SEQ ID NO:97), MOG 51-75 (SEQ ID NO:98), MOG 61-85 (SEQ ID NO:99), MOG 71-95 (SEQ ID NO:100), MOG 101-125 (SEQ ID NO:101), MOG 111-135 SEQ ID NO:102), MOG 131-155 (SEQ ID NO:103), MOG 151-175 (SEQ ID NO:105), MOG 161-185 (SEQ ID NO:106), MOG 171-195 (SEQ ID NO:107), MOG 191-215 (SEQ ID NO:108), MOG 1-25 (SEQ ID NO:109), and MOG 21-45 (SEQ ID NO:110), MOG 1-22 (SEQ ID NO:146), MOG 1-25(24C→24S) (SEQ ID NO:147), MOG 11-35 (SEQ ID NO:148), MOG 36-60 (SEQ ID NO:149), MOG 46-70 (SEQ ID NO:150), MOG 81-100 (SEQ ID NO:151), MOG 81-105 (SEQ ID NO:152), MOG 91-115 (SEQ ID NO:153), MOG 111-130 (SEQ ID NO:154), MOG 121-140 (SEQ ID NO:155), MOG 121-145 (SEQ ID NO:156), MOG 131-150 (SEQ ID NO:157), MOG 141-165 (SEQ ID NO:158), MOG 171-190 (SEQ ID NO:159), MOG 171-185 (SEQ ID NO:160), MOG 181-195 (SEQ ID NO:161), MOG 176-190 (SEQ ID NO:162), MOG 181-200 (SEQ ID NO:163), MOG 181-205 (SEQ ID NO:164), and MOG 191-210 (SEQ ID NO:104).

2. The MOG peptide of claim 1 comprising at least one T-cell epitope.

3. The MOG peptide of claim 2 comprising tandem copies of the same T cell epitope.

4. The MOG peptide of claim 2 comprising at least two T cell epitopes.

5. The MOG peptide of claim 4 wherein the T-cell epitopes are different.

6. The MOG peptide of claim 1 which is modified forming a modified peptide wherein said modified peptide retains its ability to be recognized by a human T cell specific for human MOG.

7. The MOG peptide claim 1 which is modified, the modification comprising substitution of a first amino acid for a second amino acid, the peptide so modified retaining a biological activity.

8. The MOG peptide of claim 7 which is modified, the modification comprising substitution of a first amino acid for at least a second amino acid.

9. The MOG peptide of claim 1 which is modified, the modification comprising a moiety coupled to the peptide, the moiety being selected from the group consisting of polyethyleneglycol, a moiety that enhances the solubility of the peptide, a moiety which facilitates purification of the peptide, and a moiety which comprises a proteolytic cleavage site.

10. A peptide of human MOG comprising at least one T cell epitope recognized by a human T cell specific for human MOG, said peptide selected from the group consisting of:

all or a portion of amino acids 1-95 of human MOG wherein amino acid sequences 10-13 and 46-57 of human MOG are present;

all or a portion of amino acids 111-135 of human MOG wherein amino acid sequence 111-115 of human MOG is present; and

all or a portion of amino acids 171-215 of human MOG wherein amino acid sequence 181-190 of human MOG is present,

wherein the amino acid sequence for human MOG is shown in SEQ ID NO: 2.

11. The peptide of claim 10 comprising all or a portion of amino acids 1-95 of human MOG wherein amino acid sequences 10-13 and 46-57 of human MOG are present.

12. The peptide of claim 10 comprising all or a portion of amino acids 111-135 of human MOG wherein amino acid sequence 111-115 of human MOG is present.

13. The peptide of claim 10 comprising all or a portion of amino acids 171-215 of human MOG wherein amino acid sequence 181-190 of human MOG is present.

14. The peptide of claim 10 comprising amino acids 171-195 of human MOG.

15. A composition comprising at least one peptide of human MOG comprising at least one T cell epitope recognized by a human T cell specific for human MOG, said at least one peptide selected from the group consisting of:

all or a portion of amino acids 1-95 of human MOG wherein amino acid sequences 10-13 and 46-57 of human MOG are present;

all or a portion of amino acids 111-135 of human MOG wherein amino acid sequence 111-115 of human MOG is present; and

all or a portion of amino acids 171-215 of human MOG wherein amino acid sequence 181-190 of human MOG is present,

wherein the amino acid sequence for human MOG is shown in SEQ ID NO: 2.

16. The composition of claim 15 comprising at least two peptides consisting of all or a portion of amino acids 1-95 of human MOG, wherein said at least two peptides comprise amino acid sequences 10-13 and 46-57 of human MOG and wherein the amino acid sequences 10-13 and 46-57 of human MOG are present separately or together in either or both of such at least two peptides, wherein each peptide comprises at least one T cell epitope recognized by a human T cell specific for human MOG.

17. The composition of claim 15 comprising at least two peptides consisting of all or a portion of amino acids 1-95 of human MOG, wherein said at least two peptides comprise amino acid sequences 10-13 and 46-57 of human MOG and wherein the amino acid sequences 10-13 and 46-57 of human MOG are present separately or together in either or both of such at least two peptides, and

a peptide consisting of all or a portion of amino acids 111-135 of human MOG wherein amino acid sequence 111-115 of human MOG is present,

wherein each peptide comprises at least one T cell epitope recognized by a human T cell specific for human MOG.

18. The composition of claim 15 comprising at least two peptides consisting of all or a portion of amino acids 1-95 of human MOG, wherein said at least two peptides comprise amino acid sequences 10-13 and 46-57 of human MOG and wherein the amino acid sequences 10-13 and 46-57 of human MOG are present separately or together in either or both of such at least two peptides, and

a peptide consisting of all or a portion of amino acids 171-215 of human MOG wherein amino acid sequence 181-190 is present,

wherein each peptide comprises at least one T cell epitope recognized by a human T cell specific for human MOG.

19. The composition of claim 15 comprising at least two peptides consisting of all or a portion of amino acids 1-95 of human MOG, wherein said at least two peptides comprise amino acid sequences 10-13 and 46-57 of human MOG and wherein the amino acid sequences 10-13 and 46-57 of human MOG are present separately or together in either or both of such at least two peptides, and

a peptide consisting of all or a portion of amino acids 111-135 of human MOG wherein amino acid sequence 111-115 of human MOG is present, and

a peptide consisting of all or a portion of amino acids 171-215 of human MOG wherein amino acid sequence 181-190 of human MOG is present,

wherein each peptide comprises at least one T cell epitope recognized by a human T cell specific for human MOG.

20. The composition of claim 15 comprising at least two peptides.

21. The composition of claim 16 wherein said at least two peptides are selected from the group consisting of:

MOG 1-20 (SEQ ID NO:--),
MOG 1-25 (SEQ ID NO:--),
MOG 36-60 (SEQ ID NO:--),
MOG 41-60 (SEQ ID NO:--),
MOG 41-65 (SEQ ID NO:--), and
MOG 46-70 (SEQ ID NO:--).

22. The composition of claim 21 comprised of a first peptide and a second peptide, wherein said first peptide is selected from the group consisting of:

MOG 1-20 (SEQ ID NO:--), and
MOG 1-25 (SEQ ID NO:--);

and wherein said second peptide is selected from the group consisting of:

MOG 41-60 (SEQ ID NO:--), and
MOG 41-65 (SEQ ID NO:--).

23. The composition of claim 22 further comprising a third peptide, wherein said third peptide is selected from the group consisting of:

MOG 171-195 (SEQ ID NO:--),
MOG 181-195 (SEQ ID NO:--), and
MOG 176-190 (SEQ ID NO:--).

24. The composition of claim 15 wherein said at least one peptide is selected from the group consisting of:

MOG 171-195 (SEQ ID NO:--),
MOG 181-195 (SEQ ID NO:--), and
MOG 176-190 (SEQ ID NO:--).

25. The composition of claim 15 further comprising at least one additional peptide selected from the group consisting of:

MOG 11-35 (SEQ ID NO:--),
MOG 21-40 (SEQ ID NO:--),
MOG 21-45 (SEQ ID NO:--),
MOG 31-55 (SEQ ID NO:--),
MOG 51-75 (SEQ ID NO:--),
MOG 61-85 (SEQ ID NO:--),
MOG 71-95 (SEQ ID NO:--),
MOG 81-105 (SEQ ID NO:--),
MOG 91-110 (SEQ ID NO:--),
MOG 91-115 (SEQ ID NO:--),
MOG 101-125 (SEQ ID NO:--),
MOG 121-145 (SEQ ID NO:--),
MOG 131-155 (SEQ ID NO:--),
MOG 141-160 (SEQ ID NO:--),
MOG 151-175 (SEQ ID NO:--),
MOG 161-185 (SEQ ID NO:--),
MOG 171-185 (SEQ ID NO:--),
MOG 181-205 (SEQ ID NO:--),
MOG 191-215 (SEQ ID NO:--), and
MOG 199-218 (SEQ ID NO:--).

26. The composition of claim 16 further comprising at least one additional peptide selected from the group consisting of:

MOG 11-35 (SEQ ID NO:--),
MOG 21-40 (SEQ ID NO:--),
MOG 21-45 (SEQ ID NO:--),
MOG 31-55 (SEQ ID NO:--),
MOG 51-75 (SEQ ID NO:--),
MOG 61-85 (SEQ ID NO:--),
MOG 71-95 (SEQ ID NO:--),
MOG 81-105 (SEQ ID NO:--),
MOG 91-110 (SEQ ID NO:--),
MOG 91-115 (SEQ ID NO:--),
MOG 101-125 (SEQ ID NO:--),
MOG 121-145 (SEQ ID NO:--),
MOG 131-155 (SEQ ID NO:--),
MOG 141-160 (SEQ ID NO:--),
MOG 151-175 (SEQ ID NO:--),
MOG 161-185 (SEQ ID NO:--),
MOG 171-185 (SEQ ID NO:--),
MOG 181-205 (SEQ ID NO:--),
MOG 191-215 (SEQ ID NO:--), and
MOG 199-218 (SEQ ID NO:--).

27. The composition of claim 15 further comprising an additional peptide of a human myelin protein said additional peptide comprising at least one T cell epitope recognized by a human T cell specific for said human myelin protein.

28. The composition of claim 27 wherein said human myelin protein is selected from the group consisting of MBP, MOG, MAG and PLP.

29. The composition of claim 28 wherein said human myelin protein consists of MBP.

30. The composition of claim 16 further comprising an additional peptide of a human myelin protein said additional peptide comprising at least one T cell epitope recognized by a human T cell specific for said human myelin protein.

31. The composition of claim 30 wherein said human myelin protein is selected from the group consisting of MBP, MOG, MAG and PLP.

32. The composition of claim 31 wherein said human myelin protein consists of MBP.

33. The composition of claim 17 further comprising an additional peptide of a human myelin protein said additional peptide comprising at least one T cell epitope recognized by a human T cell specific for said human myelin protein.

34. The composition of claim 33 wherein said human myelin protein is selected from the group consisting of MBP, MOG, MAG and PLP.

35. The composition of claim 34 wherein said human myelin protein consists of MBP.

36. The composition of claim 18 further comprising an additional peptide of a human myelin protein said additional peptide comprising at least one T cell epitope recognized by a human T cell specific for said human myelin protein.

37. The composition of claim 36 wherein said human myelin protein is selected from the group consisting of MBP, MOG, MAG and PLP.

38. The composition of claim 37 wherein said human myelin protein consists of MBP.

39. The composition of claim 19 further comprising an additional peptide of a human myelin protein said additional peptide comprising at least one T cell epitope recognized by a human T cell specific for said human myelin protein.

40. The composition of claim 39 wherein said human myelin protein is selected from the group consisting of MBP, MOG, MAG and PLP.

41. The composition of claim 40 wherein said human myelin protein consists of MBP.

42. A composition comprising a MOG peptide of claim 1.

43. The composition of claim 42, wherein the peptide comprises at least one T cell epitope.

44. A composition comprising at least two peptides of human MOG, at least one of which is a peptide of claim 1.

45. The composition of claim 42 further comprising a peptide of human myelin basic protein.

46. The composition of claim 43 further comprising a peptide of human myelin basic protein.

47. A composition comprising tandem copies of a MOG peptide of claim 1.

48. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising a peptide of claim 1 and a pharmaceutically acceptable carrier.

49. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising a peptide of claim 10 and a pharmaceutically acceptable carrier.

50. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising the composition of claim 15 and a pharmaceutically acceptable carrier.

51. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising the composition of claim 16 and a pharmaceutically acceptable carrier.

52. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising the composition of claim 17 and a pharmaceutically acceptable carrier.

53. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising the composition of claim 18 and a pharmaceutically acceptable carrier.

54. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising the composition of claim 19 and a pharmaceutically acceptable carrier.

55. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising the composition of claim 21 and a pharmaceutically acceptable carrier.

56. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising the composition of claim 22 and a pharmaceutically acceptable carrier.

57. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising the composition of claim 23 and a pharmaceutically acceptable carrier.

58. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising the composition of claim 25 and a pharmaceutically acceptable carrier.

59. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising the composition of claim 27 and a pharmaceutically acceptable carrier.

60. A multipeptide therapeutic composition for treating multiple sclerosis in a mammal comprising the composition of claim 42 and a pharmaceutically acceptable carrier.

61. A multipeptide therapeutic composition for treating multiple sclerosis in a mammal comprising the composition of claim 43 and a pharmaceutically acceptable carrier.

62. A multipeptide therapeutic composition for treating multiple sclerosis in a mammal comprising the composition of claim 44 and a pharmaceutically acceptable carrier.

63. A multipeptide therapeutic composition for treating multiple sclerosis in a mammal comprising the composition of claim 45 and a pharmaceutically acceptable carrier.

64. A multipeptide therapeutic composition for treating multiple sclerosis in a mammal comprising the composition of claim 46 and a pharmaceutically acceptable carrier.

65. A method of treating multiple sclerosis in a mammal comprising the step of administering to the mammal the composition of claim 48 in an amount sufficient to down-regulate an autoimmune response in the mammal.

66. The method of claim 65 wherein the administering step is selected from the group consisting of intravenous injection, subcutaneous injection, intramuscular injection, oral administration, inhalation, sublingual administration, transdermal administration, and rectal administration.

67. The method of claim 66 wherein the composition is administered subcutaneously in non-immunogenic form in an amount sufficient to down-regulate the autoimmune response in the mammal.

68. A method of treating multiple sclerosis in a mammal comprising the step of administering to the mammal the composition of claim 60 in an amount sufficient to down-regulate the autoimmune response in the mammal.

69. The method of claim 68 wherein the administering step is selected from the group consisting of intravenous injection, subcutaneous injection, intramuscular injection, oral administration, inhalation, sublingual administration, transdermal administration, and rectal administration.

70. The method of claim 69 wherein the composition is administered subcutaneously in non-immunogenic form in an amount sufficient to down-regulate the autoimmune response in the mammal.

71. A method of treating multiple sclerosis in a mammal comprising the step of administering to the mammal the composition of claim 61 in an amount sufficient to down-regulate the autoimmune response in the mammal.

72. The method of claim 71 wherein the administering step is selected from the group consisting of intravenous injection, subcutaneous injection, intramuscular injection, oral administration, inhalation, sublingual administration, transdermal administration, and rectal administration.

73. The method of claim 72 wherein the composition is administered subcutaneously in non-immunogenic form in an amount sufficient to down-regulate the autoimmune response in the mammal.

74. A method of treating multiple sclerosis in a mammal comprising the step of administering to the mammal the composition of claim 62 in an amount sufficient to down-regulate the autoimmune response in the mammal.

75. The method of claim 74 wherein the administering step is selected from the group consisting of intravenous injection, subcutaneous injection, intramuscular injection, oral administration, inhalation, sublingual administration, transdermal administration, and rectal administration.

76. The method of claim 75 wherein the composition is administered subcutaneously in non-immunogenic form in an amount sufficient to down-regulate the autoimmune response in the mammal.

77. A method of treating multiple sclerosis in a mammal comprising the step of administering to the mammal the composition of claim 63 in an amount sufficient to down-regulate the autoimmune response in the mammal.

78. The method of claim 77 wherein the administering step is selected from the group consisting of intravenous injection, subcutaneous injection, intramuscular injection, oral administration, inhalation, sublingual administration, transdermal administration, and rectal administration.

79. The method of claim 78 wherein the composition is administered subcutaneously in non-immunogenic form in an amount sufficient to down-regulate the autoimmune response in the mammal.

80. A method of treating multiple sclerosis in a mammal comprising the step of administering to the mammal the composition of claim 64 in an amount sufficient to down-regulate the autoimmune response in the mammal.

81. The method of claim 80 wherein the administering step is selected from the group consisting of intravenous injection, subcutaneous injection, intramuscular injection, oral administration, inhalation, sublingual administration, transdermal administration, and rectal administration.

82. The method of claim 81 wherein the composition is administered subcutaneously in non-immunogenic form in an amount sufficient to down-regulate the autoimmune response in the mammal.

83. A method of treating multiple sclerosis in a mammal comprising administering to said mammal the therapeutic composition of claim 49 in an amount sufficient to down-regulate an autoimmune response in the mammal.

84. A method of treating multiple sclerosis in a mammal comprising administering to said mammal the therapeutic composition of claim 50 in an amount sufficient to down-regulate an autoimmune response in the mammal.

85. A method of treating multiple sclerosis in a mammal comprising administering to said mammal the therapeutic composition of claim 51 in an amount sufficient to down-regulate an autoimmune response in the mammal.

86. A method of treating multiple sclerosis in a mammal comprising administering to said mammal the therapeutic composition of claim 52 in an amount sufficient to down-regulate an autoimmune response in the mammal.

87. A method of treating multiple sclerosis in a mammal comprising administering to said mammal the therapeutic composition of claim 53 in an amount sufficient to down-regulate an autoimmune response in the mammal.

88. A method of treating multiple sclerosis in a mammal comprising administering to said mammal the therapeutic composition of claim 54 in an amount sufficient to down-regulate an autoimmune response in the mammal.

89. A method of treating multiple sclerosis in a mammal comprising administering to said mammal the therapeutic composition of claim 55 in an amount sufficient to down-regulate an autoimmune response in the mammal.

90. A method of treating multiple sclerosis in a mammal comprising administering to said mammal the therapeutic composition of claim 56 in an amount sufficient to down-regulate an autoimmune response in the mammal.

91. A method of treating multiple sclerosis in a mammal comprising administering to said mammal the therapeutic composition of claim 57 in an amount sufficient to down-regulate an autoimmune response in the mammal.

92. A method of treating multiple sclerosis in a mammal comprising administering to said mammal the therapeutic composition of claim 58 in an amount sufficient to down-regulate an autoimmune response in the mammal.

93. A method of treating multiple sclerosis in a mammal comprising administering to said mammal the therapeutic composition of claim 59 in an amount sufficient to down-regulate an autoimmune response in the mammal.

94. An isolated peptide comprising at least two regions each having human T cell stimulating activity, said regions each comprising at least one T cell epitope of a human myelin protein, said epitope recognized by a T cell specific for said human myelin protein.

95. An isolated peptide of claim 94 wherein said human myelin protein consists of MOG.

96. An isolated peptide of claim 94 wherein the regions are arranged in a configuration different from a naturally-occurring configuration of the regions in the human myelin protein.

97. An isolated peptide of claim 95 wherein the regions are selected from the group consisting of:

MOG 1-20 (SEQ ID NO:--),
MOG 1-25 (SEQ ID NO:--),
MOG 11-35 (SEQ ID NO:--),
MOG 21-40 (SEQ ID NO:--),
MOG 21-45 (SEQ ID NO:--),
MOG 31-55 (SEQ ID NO:--),
MOG 36-60 (SEQ ID NO:--),
MOG 41-60 (SEQ ID NO:--),
MOG 41-65 (SEQ ID NO:--),
MOG 46-70 (SEQ ID NO:--),
MOG 51-75 (SEQ ID NO:--),
MOG 61-85 (SEQ ID NO:--),
MOG 71-95 (SEQ ID NO:--),
MOG 81-105 (SEQ ID NO:--),
MOG 91-110 (SEQ ID NO:--),
MOG 91-115 (SEQ ID NO:--),
MOG 101-125 (SEQ ID NO:--),
MOG 121-145 (SEQ ID NO:--),
MOG 131-155 (SEQ ID NO:--),
MOG 141-160 (SEQ ID NO:--),
MOG 151-175 (SEQ ID NO:--),
MOG 161-185 (SEQ ID NO:--),
MOG 171-185 (SEQ ID NO:--),
MOG 171-195 (SEQ ID NO:--),
MOG 176-190 (SEQ ID NO:--),
MOG 181-195 (SEQ ID NO:--),
MOG 181-205 (SEQ ID NO:--),
MOG 191-215 (SEQ ID NO:--), and
MOG 199-218 (SEQ ID NO:--).

98. An isolated peptide of claim 94 including a proteolytic site inserted between at least two of said regions.

99. An isolated peptide of claim 95 including a proteolytic site inserted between at least two of said regions.

100. An isolated peptide of claim 97 including a proteolytic site inserted between at least two of said regions.

101. A peptide of human MBP comprising at least one T cell epitope recognized by a human T cell specific for human MBP, said peptide having an amino acid sequence selected from the group consisting of: MBP-1 (11-30) (SEQ ID NO:--), MBP-1.1 (11-29) (SEQ ID NO:--), MBP 1.2 (11-31) (SEQ ID NO:--), MBP-2 (83-105) (SEQ ID NO:--), MBP-2.1 (82-105) (SEQ ID NO:--), MBP-2.2 (82-104) (SEQ ID NO:--), MBP-2.3 (80-98) (SEQ ID NO:--), MBP-2.4 (82-102) (SEQ ID NO:--), MBP-2.5 (80-104) (SEQ ID NO:--), MBP-2.6 (80-102) (SEQ ID NO:--), MBP-3 (111-130) (SEQ ID NO:--), MBP-3.1 (111-129) (SEQ ID NO:--), MBP-4 (141-165) (SEQ ID NO:--), and MBP-5 (101-125) (SEQ ID NO:--).

102. A composition comprising at least one peptide of human MBP comprising at least one T cell epitope recognized by a human T cell specific for human MBP, said at least one peptide selected from the group consisting of: MBP-1 (11-30) (SEQ ID NO:--), MBP-1.1 (11-29) (SEQ ID NO:--), MBP 1.2 (11-31) (SEQ ID NO:--), MBP-2 (83-105) (SEQ ID NO:--), MBP-2.1 (82-105) (SEQ ID NO:--), MBP-2.2 (82-104) (SEQ ID NO:--), MBP-2.3 (80-98) (SEQ ID NO:--), MBP-2.4 (82-102) (SEQ ID NO:--), MBP-2.5 (80-104) (SEQ ID NO:--), MBP-2.6 (80-102) (SEQ ID NO:--), MBP-3 (111-130) (SEQ ID NO:--), MBP-3.1 (111-129) (SEQ ID NO:--), MBP-4 (141-165) (SEQ ID NO:--), and MBP-5 (101-125) (SEQ ID NO:--).

103. The composition of claim 102 further comprising an additional peptide of a human myelin protein said additional peptide comprising at least one T cell epitope recognized by a human T cell specific for said human myelin protein.

104. The composition of claim 103 wherein said human myelin protein is selected from the group consisting of MBP, MOG, MAG and PLP.

105. The composition of claim 104 wherein said human myelin protein consists of MOG.

106. The composition of claim 103 wherein said additional peptide of a human myelin protein is selected from the group consisting of:

MOG 1-20 (SEQ ID NO:--),
MOG 1-25 (SEQ ID NO:--),
MOG 11-35 (SEQ ID NO:--),
MOG 21-40 (SEQ ID NO:--),
MOG 21-45 (SEQ ID NO:--),
MOG 31-55 (SEQ ID NO:--),
MOG 36-60 (SEQ ID NO:--),
MOG 41-60 (SEQ ID NO:--),
MOG 41-65 (SEQ ID NO:--),
MOG 46-70 (SEQ ID NO:--),
MOG 51-75 (SEQ ID NO:--),
MOG 61-85 (SEQ ID NO:--),
MOG 71-95 (SEQ ID NO:--),
MOG 81-105 (SEQ ID NO:--),
MOG 91-110 (SEQ ID NO:--),
MOG 91-115 (SEQ ID NO:--),
MOG 101-125 (SEQ ID NO:--),
MOG 121-145 (SEQ ID NO:--),
MOG 131-155 (SEQ ID NO:--),
MOG 141-160 (SEQ ID NO:--),
MOG 151-175 (SEQ ID NO:--),
MOG 161-185 (SEQ ID NO:--),
MOG 171-185 (SEQ ID NO:--),
MOG 171-195 (SEQ ID NO:--),
MOG 176-190 (SEQ ID NO:--),
MOG 181-195 (SEQ ID NO:--),
MOG 181-205 (SEQ ID NO:--),
MOG 191-215 (SEQ ID NO:--), and
MOG 199-218 (SEQ ID NO:--).

107. A therapeutic composition for treatment of multiple sclerosis in a mammal comprising a peptide of claim 101 and a pharmaceutically acceptable carrier.

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1                                                31
GAA TTC CGG GGG GCT GTT TCT GCG GGC ACA GCT GCA GCA ATT ACC GGA GTG GAG GCA GGG
61                                               91
CCA GGC AGC ACT GCC TCC AAG ATC TTC CCT TGG GCT TTT CAG CAG TAA GGG GAC ATG CAC
121                                              151
CCC AAG GGC CTC CAC TTG GCC TGA CCT TGC TGC GGG GGC TCT CTG TCC CCA GGA ACA GTA
181                                              211
GAG ATG GCA AGC TTA TCA AGA CCC TCT CTG CCC AGC TGC CTC TGC TCC TTC CTC CTC CTC
    met ala ser leu ser arg pro ser leu pro ser cys leu cys ser phe leu leu leu
241                                              271                          10
CTC CTC CTC CAA GTG TCT TCC AGC TAT GCA GGG CAG TTC AGA GTG ATA GGA CCA AGA CAC
leu leu leu gln val ser ser ser tyr ala gly gln phe arg val ile gly pro arg his
301                                              331                          30
CCT ATC CGG GCT CTG GTC GGG GAT GAA GTG GAA TTG CCA TGT CGC ATA TCT CCT GGG AAG
pro ile arg ala leu val gly asp glu val glu leu pro cys arg ile ser pro gly lys
361                                              391                          50
AAC GCT ACA GGC ATG GAG GTG GGG TGG TAC CGC CCC CCC TTC TCT AGG GTG GTT CAT CTC
asn ala thr gly met glu val gly trp tyr arg pro pro phe ser arg val val his leu
421                                              451                          70
TAC AGA AAT GGC AAG GAC CAA GAT GGA GAC CAG GCA CCT GAA TAT CGG GGC CGG ACA GAG
tyr arg asn gly lys asp gln asp gly asp gln ala pro glu tyr arg gly arg thr glu
481                                              511                          90
CTG CTG AAA GAT GCT ATT GGT GAG GGA AAG GTG ACT CTC AGG ATC CGG AAT GTA AGG TTC
leu leu lys asp ala ile gly glu gly lys val thr leu arg ile arg asn val arg phe
```

FIG. 1

```
541                                          571                                          110
TCA GAT GAA GGA GGT TTC ACC TGC TTC TTC CGA GAT CAT TCT TAC CAA GAG GAG GCA GCA
ser asp glu gly gly phe thr cys phe phe arg asp his ser tyr gln glu glu ala ala

601                                          631                                          130
ATG GAA TTG AAA GTA GAA GAT CCT TTC TAC TGG GTG AGC CCT GGA GTG CTG GTT CTC CTC
met glu leu lys val glu asp pro phe tyr trp val ser pro gly val leu val leu leu

661                                          691                                          150
GCG GTG CTG CCT GTG CTC CTC CTG CAG ATC ACT GTT GGC CTC GTC TTC CTC TGC CTG CAG
ala val leu pro val leu leu leu gln ile thr val gly leu val phe leu cys leu gln

721                                          751                                          170
TAC AGA CTG AGA GGA AAA CTT CGA GCA GAG ATA GAG AAT CTC CAC CGG ACT TTT GAT CCC
tyr arg leu arg gly lys leu arg ala glu ile glu asn leu his arg thr phe asp pro

781                                          811                                          190
CAC TTT CTG AGG GTG CCC TGC TGG AAG ATA ACC CTG TTT GTA ATT GTG CCG GTT CTT GGA
his phe leu arg val pro cys trp lys ile thr leu phe val ile val pro val leu gly

841                                          871                                          210
CCC TTG GTT GCC TTG ATC ATC TGC TAC AAC TGG CTA CAT CGA AGA CTA GCA GGG CAA TTC
pro leu val ala leu ile ile cys tyr asn trp leu his arg arg leu ala gly gln phe

901                         218              931
CTT GAA GAG CTA CGA AAT CCC TTC TGA GTG ATG TCA CAT CTT GGC AGG GGT GGA GGA GAG
leu glu glu leu arg asn pro phe OPA

961                                          991
CCT GGT TGC CCA GGG ATT TGT CCT TGG GGA CAT CTC ATC CAT CAA GTT GCA CAC TCA CTG

1021                                         1051
GCA TCT TTG CTA TGG GGA CAT TCC AAT TTG CAC TTT CAG GAA CAC TCT GAA TTC CAA GTA
```

FIG. 1 (CONT.)

PEPTIDE (AMINO ACID NOS:)	SEQENCE (H→NH_2)	SEQ ID NO:	DRB1*1501 IC50(nM)	DRB5*0101 IC50(nM)
HUMAN MOG 1-13	GQFRVIGPRHPIR	42	93	17
HUMAN MOG 3-15	FRVIGPRHPIRAL	43	5857	198
HUMAN MOG 4-16	RVIGPRHPIRALV	44	32656	8037
HUMAN MOG 10-22	HPIRALVGDEVEL	4	100000	100000
HUMAN MOG 20-32	VELPCRISPGKNA	5	6355	7635
HUMAN MOG 24-36	CRISPGKNATGME	45	1432	69428
HUMAN MOG 33-45	TGMEVGWYRPPFS	46	47500	100000
HUMAN MOG 35-47	MEVGWYRPPFSRV	6	2257	135
HUMAN MOG 37-49	VGWYRPPFSRVVH	47	7705	479
HUMAN MOG 38-50	GWYRPPFSRVVHL	7	118	246
HUMAN MOG 42-54	PPFSRVVHLYRNG	48	13124	17
HUMAN MOG 46-58	RVVHLYRNGKDQD	49	100000	59017
HUMAN MOG 63-75	PEYRGRTELLKDA	50	5411	66
HUMAN MOG 70-82	ELLKDAIGEGKVT	51	2890	1970
HUMAN MOG 70-82,A78	ELLKDAIGAGKVT	8	587	501
HUMAN MOG 74-86	DAIGEGKVTLRIR	52	31000	975
HUMAN MOG 74-86,A78	DAIGAGKVTLRIR	53	5008	247
HUMAN MOG 88-100,K89,S98	VKFSDEGGFTSFF	9	137	185
HUMAN MOG 94-106	GGFTCFFRDHSYQ	54	11150	7389
HUMAN MOG 103-115	HSYQEEAAMELKV	55	1201	697

FIG. 2

PEPTIDE (AMINO ACID NO:)	SEQUENCE (H→NH_2)	SEQ ID NO:	DRB1*1501 IC50(nM)	DRB5*0101 IC50(nM)
HUMAN MOG 117-129	DPFYWVSPGVLVL	11	12840	83
HUMAN MOG 118-130	PFYWVSPGVLVLL	56	534	238
HUMAN MOG 127-139	LVLLAVLPVLLLQ	57	1708	467
HUMAN MOG 134-146	PVLLLQITVGLVF	58	17725	8753
HUMAN MOG 140-152	ITVGLVFLCLQYR	59	1260	786
HUMAN MOG 142-154	VGLVFLCLQYRLR	60	1735	75
HUMAN MOG 143-155	GLVFLCLQYRLRG	61	907	240
HUMAN MOG 144-156	LVFLCLQYRLRGK	62	747	119
HUMAN MOG 147-159	LCLQYRLRGKLRA	63	7677	50
HUMAN MOG 149-161	LQYRLRGKLRAEI	64	1443	139
HUMAN MOG 155-167	GKLRAEIENLHRT	65	2803	178
HUMAN MOG 166-178	RTFDPHFLRVPCW	66	1844	43
HUMAN MOG 170-182	PHFLRVPCWKITL	169	100000	100000
HUMAN MOG 181-193	ITLFVIVPVLGPL	16	1029	100000
HUMAN MOG 190-202	GPLVALIICYNWL	67	693	498
HUMAN MOG 193-205	VALIICYNWLHRR	68	16	5
HUMAN MOG 194-206	ALIICYNWLHRRL	69	404	119
HUMAN MOG 195-207	LIICYNWLHRRLA	70	1280	461
HUMAN MOG 197-209	ICYNWLHRRLAGQ	71	1571	3806
HUMAN MOG 199-211	YNWLHRRLAGQFL	72	100000	100000

FIG. 2 (CONT.)

AMINO ACID	2	3	4	5	6	7	8	9	10	11	12
A	1.0	235.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
C	2.2	6818.2	1.9	0.3	0.4	1.0	0.8	0.7	0.8	0.9	0.7
D	3.9	6818.2	7.0	2.4	0.9	1.4	7.8	4.3	2.2	26.0	1.6
E	1.4	6818.2	12.0	3.8	0.5	1.0	7.0	5.0	1.4	31.0	2.2
F	0.7	1.7	0.4	0.6	0.3	0.8	12.4	1.9	0.6	36.0	0.4
G	0.7	1570.5	4.2	0.8	2.0	1.0	1.1	2.0	1.5	1.4	1.1
H	2.4	6818.2	0.9	2.1	0.7	0.9	2.9	0.5	2.2	4.0	1.1
I	2.0	6.4	0.4	0.9	0.8	0.7	0.8	1.2	0.7	11.7	1.6
K	1.2	6818.2	0.4	2.7	30.0	1.3	13.3	20.0	1.1	23.0	1.1
L	1.5	15.4	0.4	0.5	0.6	1.8	1.4	1.0	0.9	4.2	0.8
M	1.2	8.3	1.4	0.5	0.3	0.7	3.3	0.81	0.8	4.5	0.7
N	2.0	4159.1	0.7	2.6	0.2	1.2	0.6	1.2	0.9	5.0	1.4
P	7.6	83.6	544.0	1.7	70.8	3.0	1.5	0.8	0.7	5.4	1.2
Q	0.7	6818.2	0.9	4.2	0.6	1.6	1.9	8.5	0.6	5.4	0.6
R	1.5	6818.2	0.4	2.7	18.3	1.1	17.2	14.0	1.3	18.0	1.4
S	1.5	6818.2	0.7	0.4	0.8	1.0	0.7	1.6	2.6	1.7	1.2
T	0.7	371.6	0.8	1.3	1.2	0.7	0.6	1.7	3.0	6.9	0.8
V	1.1	14.1	0.5	1.0	1.0	0.8	0.9	1.2	0.6	6.2	1.8
W	3.2	1.2	0.6	1.8	1.5	0.7	2.8	1.5	1.2	7.8	0.6
Y	1:2	1.0	0.6	0.7	0.6	0.5	3.2	0.4	0.4	10.2	0.4

FIG. 3

PEPTIDE	SEQUENCE													PREDICTED IC50(nM)	EXPERIMENTAL IC50(nM)	SEQ ID NO:
TETANUS 591-603	K	I	Y	S	Y	F	P	S	V	I	S	K	V	12	9.5	21
COMPONENT RATIO		2.0	1.0	0.7	0.7	0.3	3.0	0.7	1.2	0.7	1.7	1.1				
HA 103-115	P	D	Y	A	S	L	R	S	L	V	A	S	S	9	19	19
COMPONENT RATIO		3.9	1.0	1.0	0.4	0.6	1.1	0.7	1.0	0.6	1.0	1.2				
RMBP 90-102	H	F	F	K	N	I	V	T	P	R	T	P	P	46	40	22
COMPONENT RATIO		0.7	1.7	0.4	2.6	0.8	0.8	0.6	0.8	1.3	6.9	1.2				
HA 307-319	P	K	Y	V	K	Q	N	T	L	K	L	A	T	44	40	20
COMPONENT RATIO		1.2	1.0	0.5	2.7	0.6	1.2	0.6	1.0	1.1	4.2	1.0				
FLU NP 383-395	S	R	Y	W	A	I	R	T	R	S	G	G	I	356	140	23
COMPONENT RATIO		1.5	1.0	0.6	1.0	0.8	1.1	0.6	14.0	2.6	1.4	1.1				
TETANUS 828-840	M	Q	Y	I	K	A	N	S	K	F	I	G	I	1272	2800	26
COMPONENT RATIO		0.7	1.0	0.4	2.7	1.0	1.2	0.7	20.0	0.6	11.7	1.1				
MYOGLOBIN 67-79	T	V	L	T	A	L	G	A	I	L	K	K	K	3299	4800	29
COMPONENT RATIO		1.1	15.4	0.8	1.0	0.6	1.0	1.0	1.2	0.9	23.0	1.1				
HA 23-35	G	T	L	V	K	T	I	T	D	D	Q	I	E	7431	6700	32
COMPONENT RATIO		0.7	15.4	0.5	2.7	1.2	0.7	0.6	4.3	2.2	5.4	1.6				
TUB 19KD 2-14	H	R	V	K	R	G	L	T	V	A	V	A	G	4445	13000	31
COMPONENT RATIO		1.5	14.1	0.4	2.7	2.0	1.8	0.6	1.2	1.0	6.2	1.0				
TUB 65KD 416-428	T	L	L	Q	A	A	P	A	L	D	K	L	K	33321	52000	30
COMPONENT RATIO		1.5	15.4	0.9	1.0	1.0	3.0	1.0	1.0	2.2	23.0	0.8				
PERTUSSIS 31-43	N	V	L	D	H	L	T	G	R	S	S	Q	V	55314	95000	28
COMPONENT RATIO		1.1	15.4	7.0	2.1	0.6	0.7	1.1	14.0	2.6	1.7	0.6				
MATRIX 18-30	G	P	L	K	A	E	I	A	Q	R	L	E	D	24062	100000	27
COMPONENT RATIO		7.6	15.4	0.4	1.0	0.5	0.7	1.0	8.5	1.3	4.2	2.2				

FIG. 4

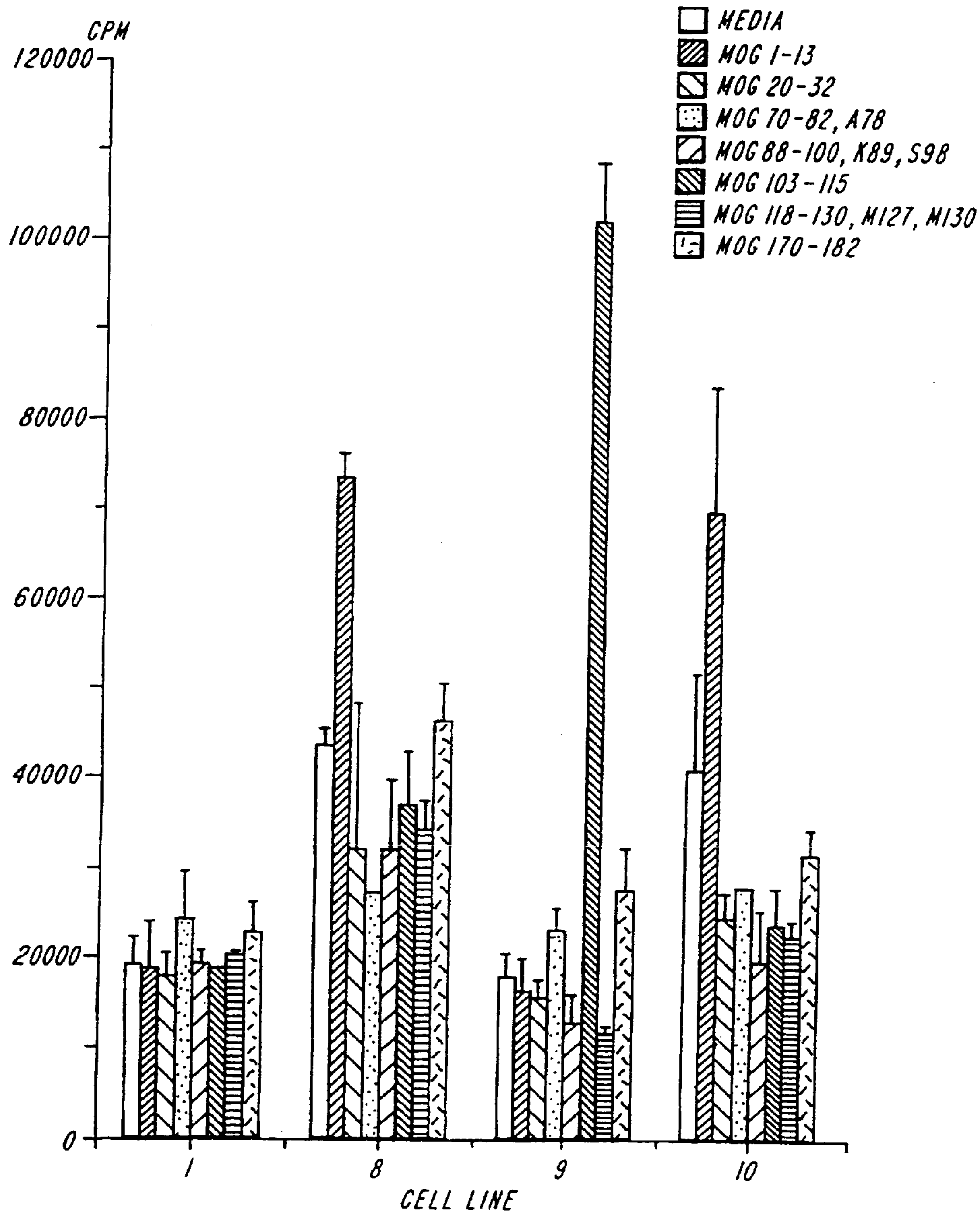
SEQ ID NO:	MOG PEPTIDE	SEQUENCE	NO:OF AMINO ACIDS
73	1-20	G Q F R V I G P R H P I R A L V G D E V	20
74	11-30	P I R A L V G D E V E L P C R I S P G K	20
75	21-40	E L P C R I S P G K N A T G M E V G W Y	20
76	31-50	N A T G M E V G W Y R P P F S R V V H L	20
77	41-60	R P P F S R V V H L Y R N G K D Q D G D	20
78	51-70	Y R N G K D Q D G D Q A P E Y R G R T E	20
79	61-80	Q A P E Y R G R T E L L K D A I G E G K	20
80	71-90	L L K D A I G E G K V T L R I R N V R F	20
82	91-110	S D E G G F T C F F R D H S Y Q E E A A	20
83	101-120	R D H S Y Q E E A A M E L K V E D P F Y	20

FIG. 5A

MOG 1-121 (SEQ ID NO:171)

G Q F R V I G P R H P I R A L V G D E V
E L P C R T S P G K N A T G M E V G W Y
R P P F S R V V H L Y R N G K D Q D G D
Q A P E Y R G R T E L L K D A I G E G K
V T L R I R N V R F S D E G G F T C F F
R D H S Y Q E E A A M E L K V E D P F Y W

FIG. 5B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100000
80000
60000
40000
20000
0
MEDIA
MOG 1-13
MOG 20-32
MOG 70-82, A78
MOG 88-100, K89, S98
MOG 103-115
MOG 118-130, M127, M130
MOG 170-182
1
8
9
10
CELL LINE

FIG. 6

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MOG MAPPING PEPTIDE SET

SEQ.ID NO:	Residues	Sequence
73	1-20	GQFRVIGPRHPIRALVGDEV
148	11-35	PIRALVGDEVELPCRISPGKNATGM
5	21-40	ELPCRISPGKNATGMEVGWY
96	31-55	NATGMEVGWYRPPFSRVVHLYRNGK
97	41-65	RPPFSRVVHLYRNGKDQDGDQAPEY
98	51-75	YRNGKDQDGDQAPEYRGRTELLKDA
99	61-85	QAPEYRGRTELLKDAIGEGKVTLRI
100	71-95	LLKDAIGEGKVTLRIRNVRFSDEGG
82	91-110	SDEGGFTCFFRDHSYQEEAA
101	101-125	RDHSYQEEAAMELKVEDPFYWVSPG
102	111-135	MELKVEDPFYWVSPGVLVLLAVLPV
103	131-155	AVLPVLLLQITVGLVFLCLQYRLRG
112	141-160	TVGLVFLCLQYRLRGKLRAE
105	151-175	YRLRGKLRAEIENLHRTFDPHFLRV
106	161-185	IENLHRTFDPHFLRVPCWKITLFVI
107	171-195	HFLRVPCWKITLFVIVPVLGPLVAL
108	191-215	PLVALIICYNWLHRRLAGQFLEELR
115	199-218	YNWLHRRLAGQFLEELRNPF

FIG. 7

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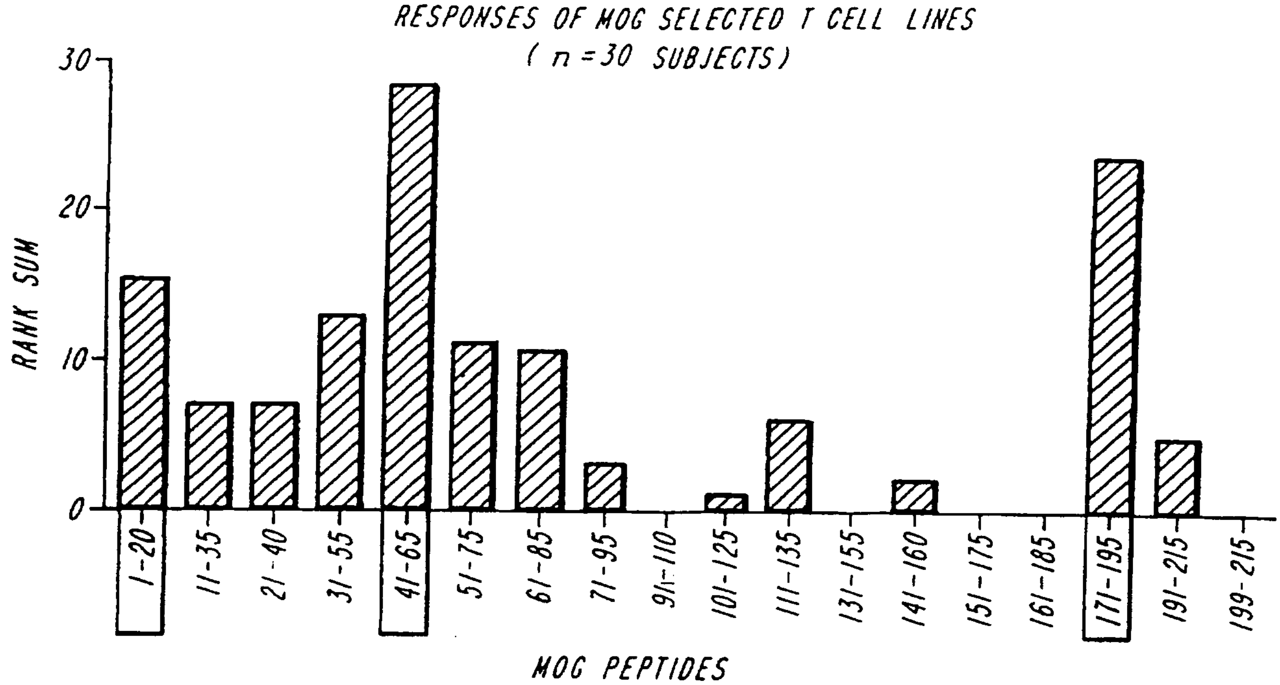

FIG. 8A

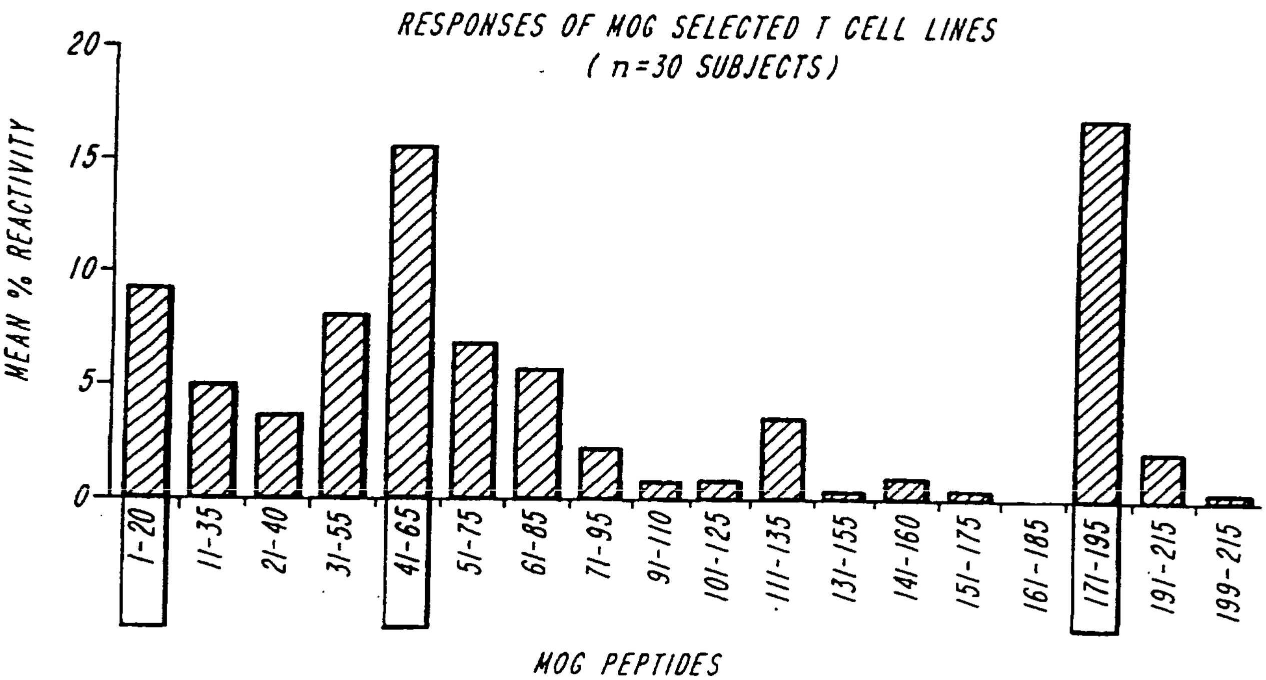

FIG. 8B

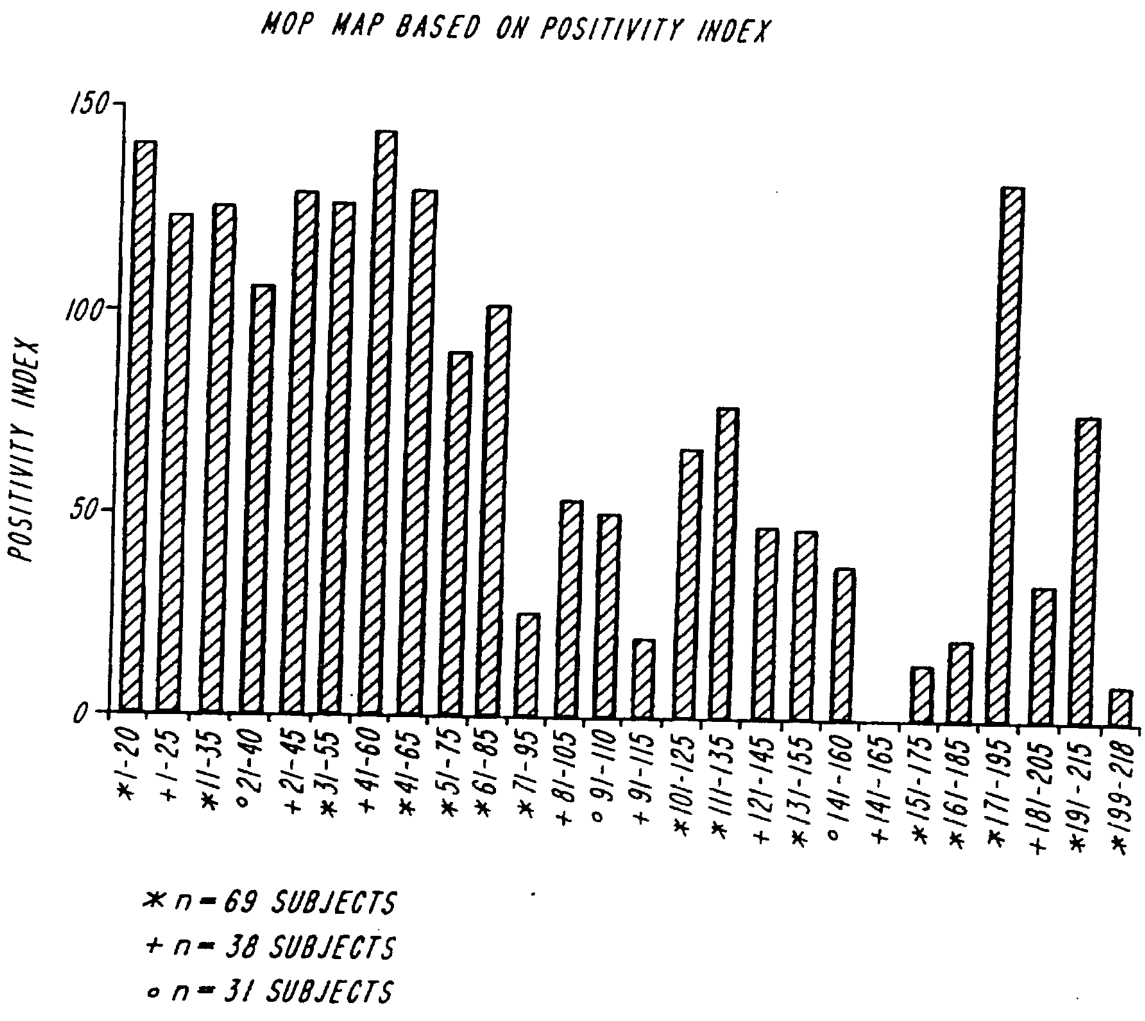
MOP MAP BASED ON POSITIVITY INDEX
POSITIVITY INDEX
150
100
50
0
*1-20
+1-25
*11-35
o21-40
+21-45
*31-55
+41-60
*41-65
*51-75
*61-85
*71-95
+81-105
o91-110
+91-115
*101-125
*111-135
+121-145
*131-155
o141-160
+141-165
*151-175
*161-185
*171-195
+181-205
*191-215
*199-218
* n = 69 SUBJECTS
+ n = 38 SUBJECTS
o n = 31 SUBJECTS

FIG. 9

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/06072

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/47 A61K38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 06727 A (IMMULOGIC PHARMA CORP ;DEVAUX BRIGITTE (US); ROTHBARD JONATHAN B () 9 March 1995 see the whole document ---	1-9, 42-44, 47,48, 60-62, 65,94,95
X	J. NEUROCHEM., vol. 63, no. 6, December 1994, UNITED STATES, pages 2353-2356, XP000614692 PHAM-DINH D ET AL.: "Characterization and expression of the cDNA coding for the human myelin/oligodendrocyte glycoprotein" see the whole document ---	1,2,4,5, 10-15, 42,94,95

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 July 1997	25.07.97

5

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chakravarty, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF IMMUNOLOGY, vol. 153, no. 3, 1994, BALTIMORE US, pages 4349-4356, XP000615802 AMOR, SANDRA ET AL: "Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice" see abstract; tables I,II ---	6-8,10, 13,15, 49,50
X	EUR. J. IMMUNOL., vol. 25, no. 4, 1995, pages 985-993, XP000614061 DE ROSBO, NICOLE KERLERO ET AL: "Chronic relapsing experimental autoimmune encephalomyelitis with a delayed onset and an atypical clinical course, induced in PL/J mice by myelin oligodendrocyte glycoprotein (MOG)-derived peptide: preliminary analysis of MOG T cell epitopes" see abstract; table 1 ---	6,10,12, 15,49,50
X	J. NEUROIMMUNOL., vol. 63, no. 1, 1 December 1995, pages 17-27, XP000613994 ADELMANN M. ET AL.: "The N-terminal domain of the myelin oligodendrocyte glycoprotein (MOG) induces acute demyelinating experimental autoimmune encephalomyelitis in the Lewis rat" see abstract; table 2 ---	1,2, 4-12,15, 48-50
X	WO 95 07096 A (UNIV TROBE ;BERNARD CLAUDE CHARLES ANDRE (AU); KERLERO DE ROSBO NI) 16 March 1995 see the whole document ---	1-6,48, 65,94,95
X	WO 95 30435 A (IMMULOGIC PHARMA CORP) 16 November 1995 see page 6, line 19 - line 32; figures 3,4 ---	94,96, 98, 101-104
X	JOURNAL OF IMMUNOLOGY, vol. 155, no. 7, 1995, BALTIMORE US, pages 3693-3699, XP000652361 MOR, FELIX; COHEN, IRUN R.: "Pathogenicity of T cells responsive to diverse cryptic epitopes of myelin basic protein in the Lewis rat" see page 3695, column 1, paragraph 3; table 1 ---	94,96, 98, 101-104

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/06072

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. EXP. MED. , vol. 179, no. 1, 1 January 1994, pages 279-290, XP000655184 WUCHERPFENNIG, KAI W. ET AL: "Structural requirements for binding of an immunodominant myelin basic protein peptide to DR2 isotypes and for its recognition by human T cell clones" see page 279, column 1, paragraph 2 - page 280, column 1, paragraph 1; tables 1-7 -----	94,96, 98, 101-104

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 96/06072

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

The present claims have been divided into two groups of invention.

Group 1 represents human MOG, including fragments and uses thereof - claims 1-93, 94(part), 95-100, 105-107.

Group 2 represents compounds other than MOG, including fragments and uses thereof - claims 94(part) and 101-104.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/06072

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9506727 A	09-03-95	AU 7725894 A	22-03-95
		CA 2170901 A	09-03-95
		EP 0716696 A	19-06-96
		FI 961000 A	04-03-96
		JP 9502346 T	11-03-97
		NO 960858 A	01-03-96
WO 9507096 A	16-03-95	AU 7646994 A	27-03-95
WO 9530435 A	16-11-95	AU 2470595 A	29-11-95
		EP 0758902 A	26-02-97
		HU 76099 A	30-06-97
		PL 317197 A	17-03-97
		ZA 9503730 A	11-01-96
		AU 4278296 A	15-05-96
		WO 9612737 A	02-05-96
		ZA 9509033 A	11-06-96